

Clinical Biochemistry

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PHILADELPHIA

TWO OF HIS PUPILS DEDICATE THIS WORK
WITH AFFECTION AND GRATITUDE TO
ALBERT P. BRUBAKER

Preface to the Third Edition

PROGRESS in biochemistry during the past five years has necessitated extensive supplementation and revision of the material presented in the last edition. The following topics are included in the major additions: the intravenous glucose tolerance test; the insulin tolerance test; present views regarding ketosis; abnormal serum globulin reactions; creatine tolerance test; acid phosphatase in prostatic malignancy; serum organic iodine in thyroid disease; intermediate metabolism of iron; tests for adrenal hypofunction and hyperfunction; sulfur metabolism; inulin, diodrast and hippuran clearance studies in the evaluation of glomerular and tubular function and renal blood flow; blood galactose in the galactose tolerance test; colloidal gold, cephalin-cholesterol and plasma prothrombin tests of hepatic function; porphyrin metabolism; quantitative studies of direct-reacting serum bilirubin; classifications of jaundice; present views regarding the physiology of gastric and pancreatic secretion; the secretin test in the study of pancreatic function; serum amylase and lipase in acute pancreatitis; new methods for the study of deficiency in vitamin A, thiamine, riboflavin, nicotinic acid, ascorbic acid and vitamin K. A new chapter has been added, dealing with "Hormone Assay and Endocrine Function," for the preparation of which the authors are deeply indebted to Dr. A. E. Rakoff. In this are considered the available chemical and biological methods for estimating the functional activity of endocrine glands.

As in previous editions, an attempt has been made to present controversial subjects in an impartial manner, supplemented by an expression of personal opinion wherever possible. There are few statements regarding the clinical significance of biochemical findings that are not supported by personal experience. Thanks are due to many friends and associates for helpful suggestions and advice, and to the publishers, W. B. Saunders Company, for their unfailing cooperation.

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(The opinions and views set forth in this book are those of the authors and are not to be considered as reflecting the policies of the Navy Department.)

Preface

MODERN advances in physiology and biochemistry have developed a need, not for another laboratory manual, but for a book designed to correlate established facts with problems encountered daily in internal medicine. The rapidity and magnitude of these developments have resulted in the growth of a highly specialized branch of laboratory medicine, namely chemical pathology. The evolution of this specialty within a specialty has unfortunately tended to remove the clinician still further from a thorough understanding of those phases of internal medicine that require the assistance of the biochemical laboratory for their complete solution.

The remarkably fruitful researches of recent years in the fields of experimental physiology and pathology, by demonstrating the significance of biochemical observations in a constantly increasing number of abnormal states, have correspondingly increased their value to clinical medicine and surgery as well as to the laboratory. Modern practice demands the application of present knowledge regarding aberrations of endocrine, renal and hepatic function, abnormalities of organic and inorganic metabolism, nutritional defects, edema, dehydration, etc., in all branches of medicine as well as in pre- and postoperative treatment. The enormous increase in the use of chemicals in industry and in the treatment of disease and the growing appreciation of the possibly deleterious effects of such agents upon the organism have also increased the service that the biochemical laboratory may render to the clinician. The intelligent employment of these facilities will be of fundamental value in the increasingly important field of industrial toxicology.

The essential function of the laboratory is to supply the clinician with information which will complement that which he may obtain by other methods. Each patient presents a problem which cannot possibly be appreciated on the basis of dissociated laboratory studies. However, in order to take full advantage of the findings of the biochemist, the clinician must have a clear understanding of the significance and limitations of the results of laboratory investigation. This must be based upon an appreciation of the biochemical and physiologic factors involved in

the preservation and alteration of organ and tissue function for it may be stated, more truly than ever before, that physiology is the handmaid of medicine.

The progress made in the fields of biochemistry, metabolism, nutrition, colloidal and physical chemistry is based largely upon observations of a highly specialized and technical nature. This often renders the original literature unavailable to the majority of students and clinicians. As a result they usually either accept the brief interpretative statements made in most works on diagnosis by laboratory methods or they rely upon the chemical pathologist for an interpretation of his findings. The position of the latter is little better than that of the clinician who is required to explain the significance of an enlarged liver in an individual whom he has not seen and concerning whose clinical history and physical condition he knows nothing. Experience in the laboratory and in the clinic has impressed the authors with the difficulty which students and physicians experience in bridging the wide gap between abstract biochemistry or physiology and clinical medicine. Books and articles in abundance have been written for and by specialists, but only a few have attempted to interpret specialized knowledge for the physician. The undergraduate student of medicine and the progressive physician wish to be familiar with the applications of biochemistry to clinical medicine and surgery. They should be as well acquainted with the limitations as with the significance of biochemical findings in any given case. This volume constitutes an attempt to supply this information.

Haldane has stated that the aim of physiology is to consider how the internal environment of the body is kept constant in spite of continual alterations in the external environment. The aim of this treatise is to consider how the internal environment of the body is altered by certain specific changes in tissue and organ physiology. It is further intended to indicate the manner in which the physician may best avail himself of information which can be obtained by biochemical studies. To this end the subject of functional diagnosis by chemical methods had been considered in considerable detail. With few exceptions, the technic of laboratory methods has not been discussed, being available in many admirable standard texts on that subject. The discussion has been restricted to those phases of biochemistry which are concerned with problems commonly encountered in clinical medicine and, therefore, purely abstract and theoretical considerations have been excluded

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Chapter I

Carbohydrate Metabolism

DIGESTION AND ABSORPTION¹

INGESTED disaccharides and polysaccharides are converted into monosaccharides by the action of enzymes present in the salivary (ptyalin), pancreatic (amylase) and intestinal (invertase, maltase, lactase) secretions. Glucose (dextrose) is the most important of these end products, levulose and galactose being formed in smaller amounts under ordinary circumstances in adults (from sucrose and lactose respectively). These monosaccharides are absorbed from the intestine and carried to the liver in the portal circulation. The processes of digestion and absorption occur gradually, the quantity of glucose reaching the liver being less than 1.8 Gm. per kilogram of body weight per hour following a carbohydrate meal.

Glucose is not absorbed in appreciable amounts from the stomach. Relatively small amounts are absorbed from the colon, particularly under conditions of normal blood sugar concentration. It also appears that the rate of absorption of glucose from the small intestine remains quite constant from hour to hour regardless, within wide limits, of the amount of sugar present and the concentration in which it was introduced. This uniform rate of absorption may be explained on the basis that soon after entering the intestine the concentration of glucose reaches a relatively constant level, apparently between 2.5 and 5.5 per cent,¹⁴ which it maintains until completely absorbed. The intestinal mucosa exhibits a highly selective action with regard to the absorption of various sugars, which is independent of the size of their molecules. The relative rates of absorption of glucose, galactose, fructose, mannose, xylose and arabinose may be designated by the figures 100, 110, 43, 19, 15 and 9, these sugars being absorbed at the same rate from the peritoneal cavity.¹⁵ It has been suggested that the relatively rapid rate of absorption of glucose, galactose and fructose is dependent upon their phosphorylation (hexose-phosphate formation) in the intestinal mucosa during the process of their absorption.^{14,16} When phosphorylation was inhibited by the administration of phlorhizin or iodoacetic acid, the rate of absorption of these hexoses

was reduced approximately to that of pentoses.^{1b} The absorption of glucose from the intestine was found to be considerably retarded in the absence of the adrenal cortex, and it has been suggested that the cortical hormone is necessary for normal phosphorylation and consequently normal absorption.^{11, 18, 11} The validity of this attractive hypothesis is, however, open to question and the observations on which it is based have been contradicted by other observers.^{12a, 20a, 47} It has been shown that the impaired absorption of glucose and other monosaccharides in adrenalectomized animals is due to abnormal salt balance and can be corrected by administration of sodium salts.^{20a} Absorption of glucose from the intestine is also influenced by the general condition of the organism, being interfered with in infections, various intoxications, prolonged undernutrition and vitamin deficiencies. It is retarded in hypothyroidism and accelerated in hyperthyroidism. These facts must be borne in mind in interpreting results of the oral glucose tolerance test (p. 7).

• LIVER

Upon reaching the liver, levulose is converted to glycogen to a somewhat greater extent than is glucose, whereas galactose is a relatively poor glycogen-former. Significant details of the intermediary metabolism of these sugars will be considered elsewhere. Dextrose is also formed from the "carbohydrate moiety" of certain amino acids resulting from protein digestion (alanine, glycine, proline, arginine, cystine, aspartic acid, glutamic acid) as well as from glycerol and perhaps fatty acids. This process, (gluconeogenesis), is stimulated by hormones of the adrenal cortex and anterior hypophysis, the influence of the latter being mediated largely, but not entirely, by the former (pp. 17, 22). There is evidence that glucose may also be formed from fat.

The *dextrose* brought to or formed in the liver may undergo the following changes:

(a) *Storage as glycogen (glycogenesis)*. Through the agency of specific enzymes, glucose is converted into glycogen by a process of polymerization and is stored, as such, in the glandular cells of the liver. A portion of the lactic acid formed in the muscle (muscular contraction) escapes reconversion into glycogen *in situ* and passes into the blood stream, from which it is removed largely by the liver. In that organ, lactic acid is reconverted into carbohydrate, probably glycogen, in which form it may be stored and from which glucose may be subsequently liberated into the blood stream. Thus, there appears to be a carbohydrate cycle between muscle and liver, muscle sending lactic acid by way of

the blood to the liver, and the liver returning glucose by way of the blood to the muscle. The liver is capable of storing 150-200 Gm. of glycogen; this constitutes a most important carbohydrate reserve which normally appears to be the sole direct source of the sugar present in the blood stream.

(b) *Utilization in the liver (glycolysis)*. A relatively small proportion of the glucose may be utilized directly in the course of metabolic activity of the liver cells. This process appears to be one of phosphorylation and transformation to triose phosphate, pyruvic acid, CO_2 and water.

(c) *Passage into systemic circulation*. The excess glucose, *i.e.*, that which cannot be stored as glycogen or utilized directly by the liver, passes into the general circulation and is carried to the tissues where it (1) is transformed into glycogen and stored as such in the muscles, (2) is utilized for energy (glycogenolysis and glycolysis), or (3) is transformed into fat.

Changes in Hepatic Glycogen. The glycogen stored in the liver serves as a source of supply of glucose for the maintenance of the sugar concentration of the blood and the glucose requirements of the tissues. The transformation of glycogen into glucose (glycogenolysis) is effected through the medium of enzyme activity and is stimulated by one or more of several external factors:

(a) *Fall in blood sugar level*. Under normal conditions, a tendency toward a decrease in the blood sugar concentration is followed by increased hepatic glycogenolysis (p. 10).

(b) *Epinephrine*. The primary effect of epinephrine appears to be acceleration of glycogenolysis in all cells. Following the administration of epinephrine there is an immediate increase in the concentration of sugar in the blood, the duration of which depends upon the state of nutrition and upon the amount injected. This increase in blood sugar is apparently due to an increased rate of mobilization of glucose from the liver (hepatic glycogenolysis), the liver glycogen content being at first decreased and later increased, the latter phenomenon occurring as a result of the excessive and prolonged liberation of glucose from muscle glycogen and secondary stimulation of insulin secretion. In addition, epinephrine apparently inhibits the oxidation of glucose in the tissues. The glycogen content of the muscles undergoes a rapid and progressive decrease during the period of epinephrine action.

(c) *Thyroxin*.

(d) *Increased Acidity*. An increase in the hydrogen ion concentration accelerates glycogenolysis, increased acidity (to pH 6.5) favoring the activity of the glycogenolytic enzyme.

(e) *Nervous Stimulation.* Stimulation of an area in the floor of the fourth ventricle ("diabetic center") results in a discharge of liver glycogen. The impulse passes down the cord, through the splanchnic nerves to the adrenals and the liver, the glycogenolytic effect being produced by the increased secretion of epinephrine and the stimulation of the sympathetic fibers passing to the liver.

(f) *Ether Anesthesia.*

(g) *Asphyxia (Increased acidity).*

TISSUES

The glucose which, being absorbed from the intestine, passes through the liver unchanged, and that which is derived from hepatic glycogenolysis, passes in the arterial blood to the tissues, where the following phenomena occur:

Glycogenesis. The muscles contain a store of glycogen comparable to that of the liver. From this is derived, by glycogenolysis, the glucose required for the metabolic activities of the tissues. As this muscle glycogen reserve is drawn upon, glucose is abstracted from the blood and is transformed into glycogen (tissue glycogenesis). Insulin is involved in this phase of carbohydrate metabolism and perhaps also in the utilization of glucose in the tissues. The function of hepatic glycogen is therefore the maintenance of the blood sugar level; that of tissue glycogen is to furnish glucose for combustion in the tissues. The latter form of glucose normally plays no direct part in the maintenance of the blood sugar concentration. According to some, the mild hyperglycemic effect of pituitrin seems to be due to insulin antagonism, *i.e.*, chiefly inhibition of glycogenesis in the tissues, but others believe that it acts in a manner similar to that of epinephrine and thyroxin (stimulation of hepatic glycogenolysis).

Glycogenolysis. As the nutritional and energy requirements of the tissues demand, stored glycogen is converted into glucose. Muscular contraction is associated with the conversion of glycogen into lactic acid, about 20 per cent of which is completely combusted into CO_2 and H_2O , the greater part of the remainder being reconverted into glycogen, either *in situ* or in the liver (p. 2). The chemistry of muscle contraction is considered elsewhere (p. 122).

NORMAL POSTABSORPTIVE BLOOD SUGAR

In the postabsorptive (fasting) state, glucose is formed constantly from glycogen in the liver (hepatic glycogenolysis) and is liberated into the circulating blood, from which it is removed in its passage through the tissues to be utilized for energy

CARBOHYDRATE METABOLISM

(glycolysis) or stored as glycogen in the muscles (glycogenesis). The fasting blood sugar level represents a dynamic balance between the rate of entrance of glucose into the blood from the liver and the rate of its removal from the blood by the tissues.¹¹ Under normal conditions, removal of glucose from the systemic circulation is influenced chiefly by (1) the energy requirement of the tissues and (2) the activity of deposition of glycogen in the muscles, which is enhanced by insulin, glycogenolysis being stimulated by epinephrine. Physiologically, hepatic glycogenolysis, with consequent entrance of glucose into the systemic circulation, is stimulated by epinephrine, thyroxin and splanchnic nerve impulses and is inhibited by insulin. Hepatic gluconeogenesis from protein and perhaps also from fat is stimulated by hormones of the adrenal cortex and anterior hypophysis, the latter probably through the medium of the former.

There is evidence¹¹ that the glycogenolytic mechanism in the liver is sensitive to relatively slight variations in the blood sugar concentration. As the latter falls, the rate of glycogenolysis increases and more glucose is liberated into the blood, while an increase in blood sugar above a certain concentration is followed by diminution in the rate of hepatic glycogenolysis with a consequent decrease in the amount of glucose entering the blood. Under normal conditions, the sensitivity of this mechanism to changes in the blood sugar level is dependent to a considerable degree upon the balance between insulin, which depresses hepatic glycogenolysis, and the anterior hypophysis and adrenal cortex, which stimulate gluconeogenesis, and, in emergency states, epinephrine, which stimulates glycogenolysis. As will be indicated subsequently (p. 37), in the presence of abnormalities in these and other factors, this sensitivity is impaired, and abnormally high or low levels of blood sugar fail to call into operation the mechanism which, under normal conditions, would prevent or correct these phenomena (depression or acceleration of hepatic glycogenolysis, respectively). According to this view, the maintenance of the normal blood sugar concentration is due to the operation of a homeostatic mechanism in the liver, the fundamental regulation of the blood sugar being an auto-regulation in which the prime mover is the blood sugar level itself (Soskin).¹¹

It is obvious that in determining the sugar concentration of venous blood one is obtaining the amount of glucose remaining after a portion has been removed during its passage through the tissues; the sugar content of arterial (capillary) blood represents the glucose supply to the tissues. Consequently the arterial-venous difference represents the degree of glucose utilization by

the tissues. In the postabsorptive state the concentration of glucose in arterial (capillary) blood is practically the same as that in venous blood. Fridenson states, "At all times glucose is being absorbed from the blood by the muscles and other tissues to be stored as glycogen, which is, in turn, continually decomposed to lactic acid and oxidized. The rate of removal is, in the resting, fasting state, however, too small to be readily distinguished. The blood sugar is as continuously replenished from the hepatic stores of glycogen, which are obtained from ingested or preformed glucose or by the transformation in the liver of other carbohydrates or protein."

Determination of Normal Blood Sugar. The establishment of normal limits for blood sugar is complicated by the presence, particularly in the red corpuscles, of certain nonsugar, copper-reducing substances, one of which is probably glutathione. The determination of blood sugar by the Benedict (1928 and 1931) reagent yields results ranging normally from 65 to 110 mg. per 100 cc., which appear to represent the most nearly true sugar values. Normal figures obtained by other commonly employed methods are:

Folin-Wu, 80-120 mg.; Folin-Wu modified, 80-110 mg.; Shaffer-Hartmann, 85-125 mg.; Hagedorn-Jensen, 95-135 mg. per 100 cc. The discrepancy depends upon the fact that the Benedict 1928 and 1931 reagents, which may be used with the Folin-Wu tungstic acid filtrate, are relatively unaffected by glutathione and other "saccharoids" whereas the reagents used in the other methods are affected by these substances. Furthermore it is now recognized that the Folin-Wu method, which is extensively employed in this country, gives results which are too high for high sugar values and too low for low values. For instance, near the 50 mg. level the results obtained are 15-18 per cent less than the true value. The use of the Benedict (1928 and 1931) reagent lessens the possibility of errors of interpretation due to this inaccuracy of method. In order to obtain true post-absorptive values the subject must be at complete mental and physical rest. Pain, emotional excitement, apprehension, fear, anger, may raise the blood sugar above the resting level, probably through the medium of excessive secretion of epinephrine, resulting in increased hepatic glycogenolysis.

Because of the fact that nonsugar reducing substances are present largely or entirely in the corpuscles, Folin and Herbert and Bourne have suggested the use of unlaked blood, that is, blood precipitated without laking the corpuscles, for the determination of the true sugar content of the blood. Benedict, however, believes that in view of the fact that methods are

now available for the accurate determination of sugar in whole blood filtrates, there is no reason for the employment of unlaked blood which is neither whole blood or blood plasma, the analysis of which eliminates from consideration certain substances concerning whose metabolic significance nothing is as yet known. It would appear advisable, therefore, to continue to employ whole blood filtrates for sugar determinations according to the method advocated by Benedict.

NORMAL ALIMENTARY REACTION (ABSORPTIVE RESPONSE) SUGAR TOLERANCE

The ingestion of glucose, starch and, to a lesser degree, other carbohydrates (levulose, galactose) and protein, is followed in the normal individual by an increase in the blood sugar concentration. The degree of elevation depends to some extent upon the amount of carbohydrate ingested but the relationship is not strictly quantitative. This alimentary reaction forms the basis for the commonly employed carbohydrate tolerance tests, several varieties of which have been devised.

Oral Glucose Tolerance Test. A specimen of venous blood is collected from the patient after a fast of at least twelve hours. Administer 1.75 Gm. of glucose per kilogram of body weight in 40-50 per cent solution, flavored, if desired, with the juice of one lemon. Specimens of blood are collected at twenty to thirty minute intervals for one and one-half to two hours. If the curve of arterial blood sugar is desired, specimens of capillary blood may be obtained from the finger or lobe of the ear simultaneously with those from the vein. The concentration of sugar in each specimen is determined, the micro method being used for capillary blood. It is also advisable to have the bladder emptied before administering the glucose and to collect urine voided during the test period and for several hours subsequently for the purpose of determining the glucose content.

The test may be simplified by administering an arbitrary amount of glucose (50-100 Gm.) and by diminishing the number of blood specimens. A fasting specimen should always be taken. Samples may then be removed hourly or a single sample may be taken at the end of two to three hours, at which time the blood sugar will, under normal conditions, have returned to its resting level.

In normal subjects, increasing the quantity of glucose ingested, from 25 to 200 Gm., has little or no significant effect upon the height to which the blood sugar rises, but it may prolong the period of hyperglycemia.

The characteristics of the normal venous blood sugar curve are as follows:

(a) The blood sugar rises sharply to reach a maximum of 40-50 mg. per 100 cc. above the fasting level within the first hour (usually thirty to forty-five minutes).

(b) A return to a normal fasting level at the end of one and one-half to two hours.

(c) A subsequent fall to a slightly subfasting concentration at about two hours (hypoglycemic phase) and a rise to the fasting level at three to four hours.

The characteristics of the normal arterial (capillary) blood sugar curve are as follows:

(a) A more rapid and more pronounced rise than that of venous blood sugar. At the peak (thirty to forty-five minutes) the arterial blood sugar is from 10-50 mg. higher than the venous blood sugar (arterial-venous difference), the average difference being about 25 mg. per 100 cc.

(b) The return to a normal level is not as rapid as in the case of venous blood sugar, the two curves converging at the resting level or slightly lower in two and one-half to three hours.

Glucose should not be present in abnormal quantity in any specimen of urine.

Intravenous Glucose Tolerance Test. The possibility of abnormalities in the glucose tolerance curve due to abnormalities of absorption from the intestine may be obviated by injecting the glucose intravenously. In normal subjects, the nature of the curve depends upon the amount of glucose injected and the duration of the period of injection. However, when relatively small amounts are injected continuously at a constant rate, the blood sugar rises to a peak and then falls steadily during the period of administration, even at times to hypoglycemic levels. A satisfactory procedure consists in the intravenous injection of 0.5 Gm. of glucose per kilogram of body weight in 20 per cent solution in distilled water by constant infusion over a period of thirty minutes.⁹⁹ Blood is withdrawn for glucose determination before the injection is begun (control specimen), at the termination of the injection (thirty-minute specimen), thirty minutes later (one-hour specimen) and at subsequent hourly intervals for periods of two to six hours, as desired.

The blood sugar rises in normal subjects to a maximum of about 200-250 mg. per 100 cc. at the end of the injection, falls steadily to a slightly subresting level at two hours and returns to the pre-injection level at three to four hours, as in the case of the oral test. The hypoglycemic phase of the curve is obtained more consistently after intravenous than after oral administra-

tion of glucose, rendering this procedure of particular value in the study of conditions accompanied by hypoglycemia or increased glucose tolerance (p. 48).

Mechanism of Production of the Normal Glucose Tolerance Curve. *Rise of Blood Sugar.* The initial rise in both arterial and venous blood sugar in the oral test is due in large measure directly to the glucose absorbed from the intestine which exceeds the capacity of the liver for removing it from the portal blood and passes into the systemic circulation.

Arterial-venous Difference. This is an expression of the rate of removal of glucose from arterial blood by the tissues, particularly the muscles, for the formation of glycogen and for oxidation. Insulin plays an important part in these processes.

Fall in Blood Sugar. This appears to be due largely to three factors:

(a) REMOVAL OF SUGAR BY THE LIVER TO FORM GLYCOGEN.

(b) REMOVAL OF SUGAR BY THE EXTRAHEPATIC TISSUES FOR GLYCOGEN FORMATION AND OXIDATION. It has been generally believed that the hyperglycemia caused by the administration of glucose stimulates the pancreas to secrete more insulin, the resulting increased storage and utilization of glucose causing the fall in blood sugar following the primary rise. The validity of this hypothesis has been questioned in recent years and evidence now available suggests that the homeostatic mechanism in the liver is of greater fundamental importance than the pancreas in this connection.^{11,81-86}

(c) DIMINISHED HEPATIC GLYCOGENOLYSIS AND GLUCONEOGENESIS. The evidence referred to indicates that under normal conditions the liver responds to an increased blood sugar level by decreasing the output of blood sugar which it has been supplying from its own sources. An apt analogy has been drawn between this regulatory mechanism and a thermostatically controlled heating system.¹¹ The liver corresponds to the furnace, the blood stream to the room, the tissues to the walls and the blood sugar concentration to the room temperature; the fuel is liver glycogen and the thermostatic regulation (*i.e.*, of hepatic glycogenolysis) is represented by the interaction of hormones of the pancreatic islets (insulin), adrenal cortex, anterior hypophysis, and, under certain circumstances, also the thyroid, adrenal medulla (epinephrine) and perhaps the gonads; radiation of heat from the room is represented by the removal of glucose from the blood for oxidation and storage as muscle glycogen. Under normal conditions, as the blood sugar (*i.e.*, room temperature) increases above the normal resting level (*e.g.*, 100 mg. per 100 cc.), hepatic glycogenolysis and gluconeogenesis (*i.e.*,

heat production by the furnace) automatically diminish, and removal of sugar for storage and oxidation in the tissues (*i.e.*, radiation of heat) is accelerated. Decrease in the blood sugar is automatically followed by increased glucose production in the liver and decreased rate of removal by the tissues. As stated by Soskin,¹¹ the glucose tolerance curve, with its hyperglycemic and hypoglycemic phases, resembles the fluctuations in temperature above and below the threshold of regulation when extra heat is introduced into the system. The nature of the curve depends upon (1) the quantity of heat (amount of sugar) introduced, (2) the sensitivity and setting of the thermostat (endocrine balance) and (3) the capacity of the furnace and the fuel supply (ability of the liver to produce sugar and the glycogen store in the liver).

The glucose tolerance tends to be somewhat lower (*i.e.*, the curve of blood sugar is higher and more prolonged) in old than in young subjects. Emotional disturbances, which tend to raise the blood sugar, may cause undue and abnormally prolonged hyperglycemia. Strenuous exercise before the ingestion of glucose may cause the blood sugar to rise to an abnormally high level, whereas strenuous exercise after ingesting the glucose may cause an abnormally marked and prolonged hypoglycemic phase in some subjects. Because of the several factors, physical, emotional, dietary and gastro-intestinal, that may influence the curve, the conditions under which the test is performed should be rigidly standardized. It should be performed in the morning, at the time of the usual breakfast, the previous diet should be unrestricted (except in frank diabetes, when the test is usually superfluous) and, except when interest is centered particularly upon the hypoglycemic phase of the curve, physical exertion and emotional excitement should be avoided during the test period. Glucose tolerance tests must often be repeated before apparently abnormal results can be interpreted satisfactorily. Moreover, false normal or borderline normal curves are obtained at times as a result of disturbances of gastric emptying and intestinal motility and absorption. In such cases the intravenous test should be employed.

The state of carbohydrate nutrition has a definite influence upon the alimentary glucose response of normal individuals. An adequate deposit of glycogen in the liver and other tissues is essential to the production of a normal response as above described. If the subject is in a state of relative carbohydrate starvation the rise in blood sugar following the ingestion of glucose will be more pronounced and its fall more delayed than under normal conditions. Conversely, if the organism is in a

state of carbohydrate saturation, *i.e.*, if a high carbohydrate meal has been taken within two to three hours of the performance of the test, the rise in blood sugar will be distinctly less marked than that described above.

It is therefore important that the subject shall have partaken of a well-rounded diet for some days prior to the performance of the test. A diet high in fat and low in carbohydrate may result in a curve typical of a state of carbohydrate starvation.⁸⁹ It is also well known that the repeated administration of glucose to normal persons at intervals of a few hours is followed by progressive lowering of the curve of alimentary hyperglycemia, *i.e.*, a progressive increase in tolerance. These observations have been generally interpreted as indicating that the rather continuous absorption of adequate amounts of glucose from the intestine maintains active secretion of insulin by the pancreas and renders the islet cells sensitive to the stimulus to increased secretion furnished by the administration of glucose in the performance of the glucose tolerance test. When carbohydrates are withdrawn from the diet, insulin secretion is diminished, the machine idles, so to speak, and the sensitivity of the islet cells to sudden stimulation is diminished. When glucose is administered subsequently, an adequate amount of insulin is not secreted promptly and removal of the absorbed glucose from the blood stream is delayed. The relatively slight hyperglycemic effect of repeated doses of glucose is generally attributed to a state of increased responsiveness on the part of the pancreas, resulting from the previous administration of glucose. This influence of previous sugar administration on subsequent dextrose tolerance curves is known as the Staub-Traugott or Hamman-Hirschman effect.^{31a}

As indicated previously, the validity of this plausible explanation has been questioned in recent years. Two other hypotheses have been advanced: (1) that the increased tolerance to glucose that follows the repeated ingestion of carbohydrate is due to an increased sensitivity of the organism to insulin rather than to an actual increase in the amount of insulin secreted;³⁹ (2) that the decreased blood sugar response that follows the repeated administration of glucose is due to a decreased output of glucose from the liver (decreased hepatic glycogenolysis) and not to increased secretion of insulin.⁸¹ The latter explanation is more in accord with recent observations.

The One Hour, Two-Dose Glucose Tolerance Test. The Staub-Traugott phenomenon, referred to above, may be described briefly as follows: Normal human subjects react to repeated doses of glucose with either hypoglycemia or little or no change in glycemia, while diabetics react with definite hyper-

glycemia. This phenomenon has been utilized as the basis for several modifications of the glucose tolerance test. One of the most widely employed of these is the one-hour, two-dose test proposed by Exton.²⁴ The test is performed as follows: Dissolve 100 Gm. of glucose in about 650 cc. of water. This solution is then flavored with lemon juice and divided into two equal doses, each containing 50 Gm. of glucose in about 15 per cent solution. In the morning after a twelve-hour fast collect blood and urine samples and give the first dose of glucose, allowing one-half minute for its ingestion. Thirty minutes later collect a second blood sample and give the second dose of glucose. Thirty minutes after the ingestion of the second dose of glucose collect blood and urine samples.

According to Exton, the characteristics of the normal response to this test are as follows: (1) A fasting blood sugar within the normal limits of the particular blood sugar method employed. (2) A rise in blood sugar which does not exceed 75 mg. in the thirty-minute sample. (3) The blood sugar in the sixty-minute sample is less than, the same as, or does not exceed the thirty-minute sample by more than 5 mg. (4) All urine samples are negative to Benedict's test. We have found that the blood sugar in the sixty-minute sample may be as much as 10 mg. higher than that in the thirty-minute sample.

The normal criteria suggested by Gould *et al.*²⁹ are: (1) Normal fasting blood sugar; (2) a half-hour level less than 50 mg. above the fasting value; (3) a one-hour value less than 30 mg. above the half-hour value. In our experience, the following criteria, suggested by Matthews *et al.*,³⁰ are the most acceptable: (1) Normal fasting blood sugar; (2) one-hour blood sugar less than 160 mg. per 100 cc.; (3) all urine specimens sugar-free.

The use of this procedure is practically restricted to the diagnosis of diabetes mellitus and, in our experience, has not been as satisfactory for routine investigation of glucose tolerance as the longer oral and intravenous tests.

As stated previously, the Staub-Traugott effect has been explained by the following hypotheses: (1) The first dose of glucose stimulates the insulin-glycogen mechanism to such activity that the normal organism is then able to deal with any amount of glucose without becoming hyperglycemic. (2) The first dose of glucose increases the sensitivity of the organism to insulin, resulting in the more rapid disposal of the second dose of glucose and a consequently lesser degree of elevation of blood sugar. (3) Under the opposing influences of the hormones of the pancreas and the pituitary and adrenal glands, the liver is an essential part of a homeostatic mechanism of blood sugar regulation.

The process of hepatic glycogenolysis, with consequent liberation of glucose into the blood stream, is, under normal conditions, extremely sensitive to changes in the blood sugar level. In the presence of a normal endocrine balance, but not necessarily an extra secretion from the pancreas, the normal liver, in response to a sudden influx of exogenous sugar, decreases the liberation of glucose into the blood stream. According to Soskin, this phenomenon does not depend upon increased mobilization of insulin from the pancreas or upon a state of increased sensitivity of the organism to insulin.

EFFECT OF OTHER SUGARS

Levulose (Fructose) Tolerance. The metabolism of levulose differs from that of glucose in that it appears to be incapable of transformation into glycogen except by the liver. The difference between the behavior of these two sugars may be illustrated by certain experimental observations.

(a) In the absence of insulin (depancreatized dogs) levulose will be stored as glycogen in the liver; glucose will not.

(b) Insulin hypoglycemia is relieved by glucose much more effectively than by levulose.

(c) Following the ingestion of levulose there is comparatively little rise in blood sugar and practically no arterial-venous difference, indicating the minor rôle of the muscles in removing this sugar from the blood.

(d) Cori has found that although levulose is absorbed from the intestine much more slowly than glucose, at the end of four hours the amount of glycogen formed in the liver by the former was 39 per cent and by the latter 17 per cent. In the absence of hepatic disease or functional insufficiency the ingestion of levulose, in tolerance doses, is followed by comparatively little elevation of blood sugar, since, after absorption, it is effectively removed from circulation by the liver where it is stored as glycogen. The levulose tolerance test has therefore been utilized as a test of the integrity of liver function. It is performed as follows (Tallerman):

A sample of blood is taken in the fasting state. Fifty grams of levulose (glucose-free) are ingested, dissolved in 200 cc. of water or lemonade. Samples of blood are withdrawn at one-half-hour intervals over a period of two hours and the sugar content of all specimens determined. A normal response is characterized by:

(1) A maximum rise of less than 30 mg. per 100 cc. above the postabsorptive level.

(2) A return to the resting level in one and one-half hours.

This procedure is probably improved if the blood levulose concentration alone is determined rather than levulose plus glucose, as above.^{28,33} Normally, the blood levulose concentration, 0-8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour after ingestion of 50 Gm. of levulose, and falls to 0-10 mg. per 100 cc. at the end of two hours.

Galactose Tolerance.^{20,22,72,72a} Galactose, like levulose, is metabolized chiefly by the liver although, according to Rowe, the glands of internal secretion have an important influence upon the tolerance of the organism for this sugar, as for glucose. The metabolism of galactose differs from that of glucose chiefly in that (a) it is absorbed from the intestine somewhat more rapidly than the latter, (b) the magnitude of its increase in the blood after ingestion is much smaller than in the case of glucose, (c) the "renal threshold" for excretion of galactose is very low and (d) its intermediary metabolism is apparently influenced much less than that of glucose by the function of organs other than the liver.

After ingestion of 40 Gm. of galactose, the blood galactose concentration normally reaches a maximum of 15-35 mg. per 100 cc. in thirty to sixty minutes, falling to zero at the end of two hours. Normal subjects may excrete up to 3 Gm. of galactose (or galactose plus glucose) in the urine during the five hours immediately following ingestion of 40 Gm. of this sugar. Intravenous injection of 1 cc. of a 50 per cent solution of galactose per kilogram of body weight is followed by its rapid disappearance from the blood stream in normal subjects, none remaining at the end of seventy-five minutes.^{2,7}

INSULIN TOLERANCE TEST⁷

The purpose of the insulin tolerance test is to determine (a) the sensitivity of the organism to insulin and (b) its responsiveness to insulin-induced hypoglycemia. Under normal conditions, the blood sugar falls to about 50 per cent of the fasting level twenty to thirty minutes after intravenous injection of 0.1 unit of insulin per kilogram of body weight. It then rises rapidly, reaching the pre-injection level in ninety to one hundred and twenty minutes. The duration of the hypoglycemia is of greater significance than its degree. The precautions that must be observed in performing this test are the same as those described in connection with the glucose tolerance test, since the response in both instances is influenced by the same factors. In cases in which an exaggerated or prolonged hypoglycemic response is anticipated (p. 49), it is advisable to provide for

prompt administration of glucose as soon as clinical manifestations of hypoglycemia develop and, at times, to use $\frac{1}{2}$ – $\frac{1}{3}$ the standard dose of insulin. In such cases it is also advisable to administer carbohydrate about three hours after the test is completed.²⁷

EPINEPHRINE TOLERANCE TEST

The increase in blood sugar which follows administration of epinephrine has been utilized as an index of the quantity and availability of glycogen in the liver. After intramuscular injection of a 1:1000 solution of epinephrine hydrochloride, the blood sugar concentration normally increases 35–45 mg. per 100 cc. in forty to sixty minutes, returning to the resting level in one and three-quarters to two hours.

PHENOMENA ASSOCIATED WITH NORMAL ALIMENTARY GLUCOSE REACTION

From the laboratory standpoint there are two significant phenomena associated with the normal alimentary glucose reaction; both are indicative of increased glucose utilization.

Decreased Serum Phosphate Concentration. The inorganic phosphate of the blood appears to be intimately related to the intermediary metabolism of glucose. It is probable that the formation of a hexose-phosphate compound is an essential step in the process of utilization of glucose in the tissues. Consequently, during this process, inorganic phosphate is withdrawn from the blood, the phosphate content of the tissues, particularly muscle, being correspondingly increased and phosphate excretion depressed. These changes in phosphate occur independently of the level of blood sugar; when increased carbohydrate utilization is induced by the administration of insulin the same phenomena are observed as when glucose is administered to a normal individual, although in the former instance the blood sugar is reduced and in the latter case it is elevated. It is obvious that the determination of serum phosphate during the performance of the glucose tolerance test may be of distinct clinical value in cases in which the interpretation of the blood sugar curve is difficult. The hypophosphatemia which normally occurs has the same theoretical significance as the arterial-venous difference; *i.e.*, integrity of pancreatic islet function and normal tissue utilization of glucose.

Following the oral administration of 100 Gm. of glucose or of 1.75 Gm. per kilogram of body weight, as in the Janney test, the serum inorganic phosphate concentration falls 1–1.5 mg. per 100 cc., reaching a minimum in about one and one-fourth to one and one-half hours, then gradually rising to obtain the

resting level in four to five hours. This period (four to five hours) represents the period of active carbohydrate utilization.

In the presence of a normal storage mechanism (insulin) this curve is produced by any agency which increases the supply of glucose to the tissues and hence by factors which stimulate hepatic glycogenolysis. These include epinephrine, thyroxin and ether anesthesia. Pituitrin, which produces hyperglycemia apparently not through increasing hepatic glycogenolysis but by inhibiting insulin activity and so depressing tissue glycogenesis, causes either no alteration in serum phosphate or, in many instances, a slight increase.

Increased Respiratory Quotient (see p. 302).^{*} An increase in the respiratory quotient above the normal resting level (0.82) is usually regarded as one of the most exact indications of carbohydrate utilization (storage and combustion). If respiratory quotient determinations are carried out simultaneously with the blood sugar tolerance test it is found that in the normal subject no significant change occurs until after the blood sugar begins to drop. At the end of one to one and one-half hours the R.Q. rises from 0.82 to 0.88-0.90, reaching 0.95 or 0.96 in about two hours and then gradually decreasing to reach the resting level in about four hours. This period, coinciding with that of hypophosphatemia, represents the period of increased carbohydrate utilization. The R.Q. is likewise influenced by the factors enumerated in the consideration of the relation of serum phosphate to carbohydrate metabolism.

THIAMINE AND CARBOHYDRATE METABOLISM

A large volume of evidence is accumulating which points to the importance of an adequate thiamine intake for the maintenance of normal carbohydrate metabolism. Although, at the present time, the importance of this factor in clinical disturbances of carbohydrate metabolism is not apparent, it may be that minor grades of such disturbance escape detection or that their relation to thiamine deficiency is not recognized. It seems pertinent, therefore, to outline briefly those findings which appear most significant and most firmly established. Many of these are concerned with changes in the metabolism of lactic acid in thiamine deficiency. The following changes have been demonstrated to occur in this state:^{1a, 66, 69}

(1) Lactic and pyruvic acids accumulate in the body in abnormal quantities, particularly after exercise, the lactic acid content of the blood and urine also increasing.

(2) Delayed resynthesis of injected lactate to glycogen, with excessive rise in blood lactic and pyruvic acids.

(3) Lowered glucose tolerance, glycogen depletion and hyperglycemia. The occurrence of hyperglycemia is denied by some and is attributed by others to nervous hyperirritability and convulsions.

(4) The appearance of pyruvic acid in the urine and its formation by and accumulation in avitaminous brain tissue during respiration in lactate solution.

(5) Increased concentration of lactic acid in the heart, which is responsible for the occurrence of bradycardia in polyneuritic rats.

(6) Increased concentration of lactic and pyruvic acids in the lower brain, which is regarded as directly responsible for the characteristic opisthotonus of polyneuritic pigeons.

(7) Diminished oxygen uptake exhibited by avitaminous pigeon brain mixed with lactic acid and glucose, an increase occurring after the addition of thiamine.

Peters believes that thiamine plays a specific rôle in the intermediary metabolism of carbohydrate. It seems probable that it is concerned as a catalyst (coenzyme) in some mechanism essential for the normal metabolism of pyruvic acid, but there is disagreement concerning the exact rôle that it plays in this connection. Some believe that it acts as a cocarboxylase, others maintain that it functions as a pyruvate oxidase, while others regard it as a coenzyme for a dehydrogenase-cocarboxylase. There seems little doubt that thiamine has a specific effect in this connection and that the reported observations are not due entirely, as believed by some, to the general disturbance of nutrition that accompanies the avitaminous state.

THE ANTERIOR HYPOPHYSIS IN CARBOHYDRATE METABOLISM^{1b,5,21,49,51,52,56}

The early observations of Houssay and Magenta that hypophysectomized dogs are unusually sensitive to insulin attracted little attention at the time but have since been amply confirmed. However, since 1929 and 1930, when Houssay and his associates showed that hypophysectomy exerts a strikingly ameliorating effect upon the diabetic manifestations of pancreatectomized toads and dogs, the intense interest which was aroused in this subject has resulted in the practically unanimous opinion that the anterior pituitary ranks in importance with the pancreas and liver as a regulator of carbohydrate metabolism. The clinical significance of the large number of experimental observations that have been made in this connection is not yet clearly apparent. However, the demonstrated fundamental importance of the anterior hypophysis in carbohydrate metabolism

makes it seem probable that alterations in functional activity of this organ do play a part in clinical disturbances of carbohydrate metabolism that has thus far escaped detection. Therefore, in spite of the fact that few of the many experimental observations have an obvious practical application at the present time, no consideration of clinical disturbances of carbohydrate metabolism can be complete without some understanding of the influence of the anterior hypophysis in this connection and the possible clinical implications of these experimental observations. The form of presentation is patterned after that of Houssay.

CARBOHYDRATE METABOLISM IN HYPOPHYSECTOMIZED ANIMALS

Blood Sugar Level. (1) Under ordinary conditions the blood sugar concentration usually remains within normal limits. However, the degree of elevation of blood sugar after ether, morphine, pilocarpine and epinephrine is often less than normal.

(2) The most significant change is the occurrence of hypoglycemia in the fasting animal, the blood sugar falling rapidly and progressively after even a few hours. This may lead to fatal hypoglycemia in the dog if glucose is not administered promptly. Similar findings have been obtained in the monkey.

(3) The administration of phlorhizin, with the consequent loss of sugar in the urine, produces rapidly progressive hypoglycemia, convulsions and death in fasting animals. This phenomenon can be prevented by the administration of anterior pituitary extracts.

(4) An abnormal degree of hypoglycemia follows the hyperglycemia produced by epinephrine or glucose.

(5) The fall in blood sugar after insulin is more rapid, more marked and more prolonged than normal, and recovery is slow and difficult. Quantities of insulin that would be well tolerated by normal animals often produce fatal hypoglycemia in the absence of the hypophysis. It appears that the hypophysectomized animal is less able to mobilize glucose to combat the hypoglycemia.

Respiratory Quotient. Studies upon the toad and the dog reveal no alteration in respiratory quotient and a normal increase in this factor following administration of glucose.

Glucose Tolerance. No constant change in glucose tolerance has been reported. Many animals show increased tolerance and many no significant change. It has been reported that while normal glucose tolerance curves may be obtained in hypophysectomized dogs, these animals do not yield the Staub-Traugott effect. It has been suggested that this phenomenon may be utilized as an index of pituitary function.

Glycogen. No constant change occurs in the glycogen content of the muscles. Although a variable effect has been observed upon the quantity of liver glycogen in different species, there is considerable evidence to suggest that the liver glycogen of hypophysectomized animals is resistant to the action of glycogenolytic stimuli, such as epinephrine. This is apparently one cause for the increased sensitivity to insulin.

The state of carbohydrate metabolism in the hypophysectomized animals may be summarized as follows: Although there is no evidence of impaired capacity for storing glycogen or utilizing carbohydrate, the ability to maintain normal blood sugar levels in the fasting state is lost and the animal is highly susceptible to the influence of agents which cause the loss of glucose from the organism or which tend to lower the blood sugar level. These phenomena may be dependent upon a diminished ability of the liver to liberate glucose into the blood stream in consequence of a state of increased resistance of liver glycogen to glycogenolytic stimuli.

The characteristic changes in carbohydrate metabolism in hypophysectomized animals appear to be due to (a) decreased hepatic gluconeogenesis from protein and possibly to (b) increased utilization of glucose in the tissues. There is also a decrease in the rate of absorption of glucose from the intestine, due apparently to diminished thyroid function (p. 2). As a result of these phenomena, the level of blood sugar is lowered in the fasting state and the sensitivity to insulin is increased.

CARBOHYDRATE METABOLISM IN HYPOPHYSECTOMIZED-DEPANCREATIZED ANIMALS

The original observation by Houssay and his associates that the manifestations of diabetes in pancreatectomized toads and dogs are prevented or alleviated by hypophysectomy has been amply confirmed in a number of species. This phase of investigation is so distinctly the work of the Argentine school that the depancreatized-hypophysectomized animal is commonly referred to as the Houssay animal. Such animals, including the dog and monkey, differ from those simply pancreatectomized in the following manner:

(1) Glycosuria and polyuria are diminished and at times absent.

(2) They survive for longer periods, with less weight loss, are less susceptible to infection and their wounds heal more rapidly.

(3) The blood sugar is maintained at lower levels and is subnormal in some instances.

(4) The Houssay animal is extremely sensitive to insulin, as is the simply hypophysectomized animal.

(5) There is less ketosis and the alkali reserve remains within normal limits.

(6) Hepatic and muscle glycogen may be normal and the respiratory quotient may exhibit an almost normal increase following ingestion of glucose.

(7) The glucose tolerance curve in some cases is less distinctly abnormal.

These animals are, however, far from normal. As stated by Young, they live "precariously balanced between hypoglycemia and diabetes; a comparatively short fast may induce fatal hypoglycemia whereas extensive carbohydrate feeding may aggravate the diabetic state." The blood sugar exhibits marked fluctuations within wide limits, responding unduly to abstinence from and administration of food.

One fact that has been demonstrated clearly as a result of observations upon Houssay animals is that the organism is capable of utilizing carbohydrate in the absence of insulin. If one adheres to the view that insulin is essential for carbohydrate utilization in the normal animal, he must be forced to the conclusion that in the pancreatectomized animal the pituitary gland acts to inhibit the utilization of sugar, this inhibition being removed when this gland also is extirpated. The relation of the adrenal cortex to the hypophysis in this connection will be referred to subsequently.

One of the most striking effects of hypophysectomy in pancreatectomized animals is the great reduction in the excessive conversion of body protein and nitrogen excretion which occurs in pancreatic diabetes. This protein-sparing effect apparently cannot be attributed entirely to the resumption of efficient carbohydrate oxidation. It would appear that the ameliorating influence of hypophysectomy upon pancreatic diabetes is due to a diminished production of glucose (decreased gluconeogenesis) from protein. There is also, perhaps, a direct inhibiting action on the formation of ketone bodies.

DIABETIC ACTION OF ANTERIOR PITUITARY EXTRACTS

It has been shown that anterior pituitary extracts may induce a diabetic condition in normal animals and aggravate it in Houssay and particularly in simply pancreatectomized animals. Pituitrin has no such effect. There seems to be a rather marked species difference in susceptibility to the effect of this principle, dogs and cats being more susceptible than rats, mice and rabbits.

The daily injection of crude anterior lobe extracts or relatively pure preparations of this diabetogenic principle is accompanied by the following phenomena: Increased resistance to insulin (glycotropic action) occurs promptly, even before the blood sugar begins to rise, which occurs on the second or third day. Hyperglycemia is followed by glycosuria, polyuria, ketonuria, hyperlipemia and diminished glucose tolerance. The respiratory quotient falls and, as in pancreatic diabetes, does not exhibit the normal rise after the ingestion of carbohydrate. The characteristic features of the response to the administration of this principle are as follows:

(1) The hyperglycemia and subsequent phenomena do not appear until the second or third day following injection.

(2) These manifestations disappear or are markedly diminished in severity during fasting periods.

(3) Storage of glycogen is increased (glycostatic action). This is the only form of diabetes in which this is known to occur.

(4) Houssay states that the action of this hormone appears to be to stimulate and facilitate production of sugar and perhaps to regulate its utilization. In excessive doses it causes hyperglycemia and decreases sugar consumption.

Permanent diabetes can be produced by repeated injection of anterior pituitary extracts.^{21,59,63} This state is accompanied by degeneration and atrophy of the islands of Langerhans (beta cells) and a decrease in insulin content of the pancreas following, in some cases, a transitory hypertrophy and hyperplasia of the islet cells and an increased insulin content. The latter has been attributed to stimulation of the island cells by a "pancreatrophic hormone,"^{62,69} but it seems likely that this effect occurs in primary and transient response to the hyperglycemia induced by the extract. With prolonged hyperglycemia induced by repeated injection, the islet cells undergo functional exhaustion, degeneration and atrophy. The validity of this hypothesis regarding the importance of the blood sugar level in the pathogenesis of the permanent diabetes and pancreatic islet degeneration and atrophy is supported by recent observations by Lukens and his associates.^{55,56} They showed that permanent diabetes induced by pituitary extracts could be prevented, with morphologic restoration of the islands of Langerhans and apparently complete metabolic recovery, if the blood sugar level was kept within normal limits by administration of either insulin or phlorhizin simultaneously with the pituitary extract.

A large part of this "diabetogenic" effect of the anterior pituitary results from stimulation of the adrenal cortex by the adrenotrophic hormone, stimulating gluconeogenesis in the liver

and probably diminishing glucose utilization in the tissues. However, the fact that anterior pituitary extracts exert a mild diabetogenic effect in adrenalectomized dogs and toads indicates also a direct action on carbohydrate metabolism not mediated by the adrenal cortex.

THE ADRENAL CORTEX IN CARBOHYDRATE METABOLISM^{1b, 49-54}

The chief metabolic functions of the adrenal cortex are exerted in the regulation of (1) electrolyte and water metabolism (p. 238) and (2) carbohydrate and protein metabolism.^{46, 52} The several steroids derived from this gland differ considerably in their effects upon these processes; *e.g.*, electrolyte and water metabolism are influenced more by desoxycorticosterone than by compounds of the corticosterone type, whereas carbohydrate metabolism is influenced but slightly by the former and markedly by the latter.^{46, 52} The functions of the adrenal cortex in the regulation of carbohydrate and protein metabolism may be indicated by summarizing the consequences of adrenalectomy and of administration of potent adrenal cortical extracts.

Adrenalectomy. The following phenomena occur in adrenalectomized animals:

(a) A tendency to develop hypoglycemia in the fasting state. This is exaggerated by factors that accelerate carbohydrate utilization, such as administration of insulin, exercise and exposure to cold.

(b) Depletion of the glycogen of the liver and, to a lesser extent, of the muscles in the fasting state. Both the blood sugar level and the glycogen stores are fairly well maintained when carbohydrate or protein are fed in adequate amounts.

(c) Adrenalectomy results in striking amelioration of the diabetes of depancreatized animals, similar to the effects of hypophysectomy. There is a marked decrease in the excretion of glucose, nitrogen and ketone bodies, and a lowering of the blood sugar concentration.⁴⁹

(d) The above phenomena are counteracted by administration of potent adrenal cortex extracts or by compounds of the corticosterone type.

Administration of Adrenal Cortical Extracts. Administration of these substances to normal fasting animals is followed by:

(a) Marked increase in liver glycogen and slight or moderate increase in blood sugar.

(b) Simultaneous increase in urine nitrogen and, in some cases, glycosuria.

It has been found that the extra carbohydrate formed under such circumstances has its origin in increased protein catabolism, *i.e.*, gluconeogenesis from protein.^{51,53} There is evidence that adrenal cortical hormones may also inhibit peripheral utilization of glucose and inhibit hepatic glycogenolysis or accelerate the conversion of glucose to glycogen.

Similarity between Effects of Anterior Pituitary and Adrenal Cortex. There are certain points of similarity in the effects of the anterior pituitary and adrenal cortex on carbohydrate and protein metabolism:^{51,53} (a) Hypoglycemia occurs in fasting hypophysectomized or adrenalectomized animals, more rapidly and more markedly in the former than in the latter.

(b) Liver glycogen is reduced in both cases, but muscle glycogen strikingly only in hypophysectomized animals.

(c) There is a high rate of utilization of carbohydrate in adrenalectomized but not in hypophysectomized animals.

(d) Both exhibit similar responses to factors which normally accelerate protein catabolism and gluconeogenesis, *e.g.*, exposure to cold, pyrogenic agents, fasting, phlorhizin, anoxemia and pancreatectomy.

(e) Both are extremely sensitive to insulin.

(f) Adrenalectomy alleviates total pancreatic diabetes to a degree comparable to that effected by hypophysectomy. The available data suggest that the "diabetogenic" activity of the anterior pituitary (p. 20) is probably mediated in part by the adrenal cortex. However, the latter also possesses functions in this connection that differ somewhat from those of the former, such as inhibition of peripheral utilization of glucose.

The present status of knowledge regarding the part played by the anterior hypophysis and the adrenal cortex in carbohydrate metabolism has been summarized because of the important place which such observations must occupy in any hypothesis regarding the pathogenesis of disturbances of carbohydrate metabolism, including diabetes mellitus. Whereas their practical application is not yet readily apparent, a clear understanding of the influence of these glands is essential to a proper understanding of many phases of normal and disturbed carbohydrate metabolism.

ABNORMALITIES OF POSTABSORPTIVE BLOOD SUGAR LEVEL

FASTING HYPERGLYCEMIA

Fasting hyperglycemia may be due to either an increased rate of entrance of glucose into the blood from the liver (increased hepatic glycogenolysis or gluconeogenesis) or a decreased

rate of removal of glucose from the blood by the tissues (decreased storage and utilization). In many clinical disorders both of these mechanisms are operative and it seems probable that persistent hyperglycemia may be dependent fundamentally upon elevation of the threshold of sensitivity of the hepatic glycogenolytic mechanism to the blood sugar, i.e., a disturbance of the homeostatic mechanism in the liver (p. 5).

Diabetes Mellitus. The fundamental fault in diabetes mellitus is apparently a deficiency in pancreatic islet secretion (insulin). Recent demonstration of the influence of hormones of the anterior hypophysis and adrenal cortex upon carbohydrate metabolism (p. 17ff.) has raised some doubt as to whether a quantitative deficiency in insulin secretion is invariably the only cause of diabetes mellitus. This problem is discussed in greater detail elsewhere (p. 332). The exact way in which insulin acts in the intermediary metabolism of carbohydrates is not definitely understood but it appears to be essential for the polymerization of glucose into glycogen in the tissues and, perhaps, in the liver, and also probably retards glycogenolysis in the liver. In the absence of an adequate amount of insulin, hepatic glycogenolysis and gluconeogenesis are increased and the rate of removal of glucose from the blood for glycogenesis in the extrahepatic tissues is decreased. The obvious consequence is an increase in the concentration of glucose in the blood. The utilization of glucose at ordinary levels of blood sugar is probably impaired, but there is evidence that at very high blood sugar levels glucose can be utilized by the tissues in the presence of insulin deficiency and even in the absence of insulin if the anterior hypophysis or adrenal cortex has been removed. These facts suggest that the fundamental mechanism underlying the hyperglycemia of diabetes mellitus is one of overproduction (excessive hepatic glycogenolysis and gluconeogenesis) rather than underutilization of glucose. On the basis of the homeostatic mechanism in the liver, one may assume that because of the disturbed endocrine balance (diminished insulin, with relative excess of anterior pituitary and adrenal cortex), the "thermostat" regulating the mechanism has been set at a higher level, allowing the blood sugar to rise to a hyperglycemic level before hepatic glycogenolysis and gluconeogenesis are depressed.²²

In large series of cases of diabetes mellitus the postabsorptive blood sugar has ranged from 70 to 1850 mg. per 100 cc. of blood. Complicating conditions such as acidosis, which should be considered a part of the disease, and hyperthyroidism, naturally tend to maintain the blood sugar at a higher level than that due to the insulin deficiency per se. Although it is true that the level

of the fasting blood sugar usually parallels the severity of the condition, such a statement cannot be made unequivocally. In early cases the fasting blood sugar may be well within normal limits; as the condition becomes more advanced values of 180-300 mg. may be obtained; in advanced cases values of 400 mg. are not uncommon and, if acidosis is marked and the patient in coma, the degree of hyperglycemia may be extreme (700 mg. or over). Figures above 600 mg. are, however, rarely observed. In interpreting figures above 200 mg. per 100 cc. it must be recalled that the Folin-Wu method yields results which are consistently too high for high sugars and that, within the normal range, it yields results 15-20 mg. higher than those obtained with the Benedict 1928 reagent. In view of this discrepancy the analytic method employed should always be recorded in borderline cases.

In some instances the postabsorptive blood sugar concentration does not afford a true index of the presence or severity of an existing diabetes. As has been stated, in mild cases the fasting blood sugar may be within normal limits and further studies, such as the glucose tolerance test, must be resorted to in order to establish the true nature of the condition. This will be discussed later in greater detail (p. 38). Fasting hyperglycemia (above 130 mg. per 100 cc.) is highly suggestive of diabetes mellitus, but other conditions must be considered. In the presence of complicating factors, particularly hyperthyroidism, the diagnostic standard must be raised. Joslin and Lahey state, "To avoid premature diabetic cures, we have raised the standard for a diagnosis of diabetes in hyperthyroidism to a blood sugar of 150 mg. per cent fasting or 200 mg. per cent or more after meals in addition to glycosuria."

It has been definitely established that the blood glucose is derived from hepatic glycogen in the absence of an exogenous supply. Removal of the liver in complete diabetic (depancreatized) animals results in the disappearance of sugar from the blood as in the cases of nondiabetic animals. Advanced acute hepatic disease with serious disturbance of the glycogenic function of the liver, occurring in a patient with diabetes, may tend to diminish the blood sugar concentration (phosphorus or arsenic poisoning, toxemias of pregnancy, acute diffuse necrosis of liver, cirrhosis, and occasionally acute catarrhal jaundice). It is interesting to note in this connection that several observers have called attention to the occurrence of hypoglycemic reactions in patients with hemochromatosis receiving insulin, and to the marked fluctuation in glucose tolerance in that condition. This peculiar state is somewhat comparable to that of the

hypophysectomized-depancreatized dog, in which the blood sugar rises higher after glucose than in the pancreatcctomized dog and hypoglycemic manifestations develop more rapidly and at a higher blood sugar level than in the hepatectomized animal. It must be emphasized that whereas the superimposition of hepatic disease occasionally is associated with a lowering of the fasting blood sugar concentration in diabetics, it usually further diminishes the already lowered glucose tolerance of these individuals and increases their resistance to insulin. A tendency toward lowering of the fasting blood sugar level occurs in undernourished individuals whose carbohydrate and protein intake has been so restricted that the available glycogen store in the liver has been depleted. Under these circumstances the postabsorptive blood sugar level may mask the severity of the diabetic condition. On the other hand, as has been indicated, hyperthyroidism, hypertension, nephritis, acidosis and acute infections, occurring in a patient with diabetes mellitus may, by their independent hyperglycemic effect, exaggerate the apparent severity of the condition. It should be realized, however, that the metabolic error in diabetes is seriously aggravated by factors which increase hepatic glycogenolysis, such as hyperthyroidism, infection and acidosis, and that their presence is of definitely adverse prognostic import.

Hyperthyroidism. Hyperthyroidism, whether due to thyroid disease (exophthalmic goiter, toxic adenoma) or temporarily induced by the administration of thyroid gland substance or thyroxin, results in a state of hypersensitiveness on the part of the liver for the conversion of glycogen into glucose. This glycogenolytic action of thyroxin may be excited directly, but it is more probable that the effect is produced by rendering the liver hypersensitive to sympathetic nerve impulses, and possibly to epinephrine, since section of the splanchnic nerves prevents the discharge of glycogen. Increased glycogenolysis and also gluconeogenesis perhaps result also from the increased rate of tissue metabolism. In the presence of an adequate supply of glycogen in the liver there is a distinct tendency toward a state of fasting hyperglycemia, the tissues being constantly subjected to the necessity of handling a superabundance of glucose. This, unless excessive, is undoubtedly compensated by increased activity of the storage mechanism and increased combustion of glucose in the tissues. Obviously, unless carbohydrates are supplied in abundance, the store of hepatic glycogen will be depleted and the blood sugar will automatically fall and may become subnormal. This perhaps accounts for the relatively infrequent incidence of fasting hyperglycemia in patients with

hyperthyroidism, which is rarely severe and which has been reported as occurring in 0.5 to 8.5 per cent of large series of cases.⁴³

The mistake should not be made of interpreting a disturbance of carbohydrate metabolism dependent upon hyperthyroidism as due to diabetes mellitus. Further details of the laboratory means of differentiating these conditions will be discussed later (pp. 38, 65, 309). The patient with hyperthyroidism is undoubtedly more prone to develop diabetes than other individuals, the combination being encountered by Joslin and Lahey⁴⁴ in 1.44 per cent of 4917 true diabetics and by Regan and Wilder⁷¹ in 3.2 per cent of patients with hyperthyroidism. In an additional 4.2 per cent some degree of thyroid enlargement was present without definite evidence of hyperthyroidism.

Increased Secretion of Epinephrine. The hepatic glycogenolytic action of epinephrine has been referred to previously. If the glycogen content of the liver is adequate the injection of epinephrine is followed by a rise in blood sugar. This fact has been utilized as a test of the glycogen storing capacity of the liver. If 10 minims of a 1:1000 solution of epinephrine hydrochloride are injected intramuscularly, the blood sugar rises 35-45 mg. per 100 cc. in three-fourths to one hour and returns to the resting level in one and three-fourths to two hours.

This effect of epinephrine is probably involved in several physiologic and pathologic states.

(a) As indicated above, it appears to be a factor in the production of the hyperglycemic response to increased thyroid activity.

(b) Cannon believes that hypoglycemia, produced characteristically by the administration of insulin, results in increased sympathetic activity and increased epinephrine secretion with consequent mobilization of sugar from the liver. As Lusk states, this "arrangement represents another remarkable example of automatic adjustment when a disturbance threatens the equilibrium of the organism."

(c) Cannon and his associates have shown that various emotional states such as anger, anxiety and fear are associated with an increased quantity of epinephrine in the blood. These states, as well as conditions of mental stress, excitement and excessive cold have been found to be accompanied by an increase in blood sugar, apparently the result of the mobilization of liver glycogen by epinephrine. Pain and discomfort perhaps act in a similar manner. These conditions constitute what may be termed psychic hyperglycemia.

(d) Increased epinephrine secretion is a factor in the production of hyperglycemia in the so-called "diabetic picture" and in intracranial disorders (concussion, brain tumor, fracture of the skull and intracranial hemorrhage, with increased intracranial pressure), which cause the transmission of impulses through the splanchnic nerves to the adrenals and liver. It has also been observed in association with lesions in the region of the fourth ventricle and the hypothalamus, particularly the pons. Hyperglycemia may occur in a variety of convulsive states, including idiopathic epilepsy, Jacksonian epilepsy, tetanus, tetany, eclampsia and hypertensive encephalopathy, if the hepatic glycogen stores are adequate. If the latter are depleted, the blood sugar does not rise and indeed may fall if the convulsive state is prolonged.

(e) Fasting hyperglycemia, either constant or temporary and occurring periodically, has been observed in association with certain tumors of the adrenal medulla (pheochromocytomas), presumably dependent upon the periodic outpouring of excessive quantities of epinephrine into the blood stream. There is a rather characteristic clinical picture of paroxysmal hypertension with simultaneous paroxysms of hyperglycemia. The blood sugar concentration in the intervening periods may be normal and the hyperglycemia (and hypertension) tends to be inhibited by administration of ergotamine, which counteracts the effect of epinephrine.

Adrenal Cortical Hyperfunction. This condition, resulting from hyperplasia or tumor of the adrenal cortex, may result in (a) the adrenogenital syndrome or (b) Cushing's syndrome. The former is seldom accompanied by abnormality in carbohydrate metabolism; the latter is usually characterized by the gradual development of diminished glucose tolerance, followed by progressive elevation of the fasting blood sugar concentration, which may, however, remain normal for a long time. The hyperglycemia, when it develops, is rather resistant to insulin, and is probably due chiefly to increased hepatic gluconeogenesis.

Hyperpituitarism. The hyperglycemic effect of anterior pituitary extracts has been considered previously (p. 20). Two clinical hyperpituitary states may at times be accompanied by hyperglycemia, *i.e.*, acromegaly (eosinophilic adenoma) and the type of Cushing's syndrome due to pituitary basophilism or basophilic adenoma. The fasting blood sugar is usually normal in patients with acromegaly, particularly in the early stages of the disease, before the tumor has encroached extensively upon the remainder of the gland. In one series of 100 cases,^{18,19} 12 per cent had moderate and 12 per cent mild fasting

hyperglycemia, but many more had a diminished glucose tolerance. Coggeshall and Root¹⁴ reported the occurrence of diabetes mellitus in 17 per cent of 153 acromegalics, the former condition developing within fifteen years of the onset of acromegaly in the majority of instances (average 9.5 years). In several instances we have observed a fall in a previously elevated blood sugar, with improvement in glucose tolerance, as the pituitary tumor increased in size. The inconsistent occurrence of hyperglycemia has been explained as due to the action of excessive secretion of growth hormone in counteracting the gluconeogenic influence of the excessive secretion of the adrenocorticotrophic hormone.²⁷

Cushing's syndrome due to pituitary basophilism may be accompanied by manifestations of disturbed carbohydrate metabolism described in connection with adrenal cortical hyperfunction. These phenomena are probably dependent largely upon increased hepatic gluconeogenesis resulting from excessive adrenocorticotrophic stimulation of the adrenal cortex. The effect may in part, however, be exerted by an anterior pituitary hormone acting in some manner other than through the adrenal cortex (p. 21).

Anesthesia, Asphyxia, Hypnosis. The exact cause of the acidosis of ether and chloroform anesthesia is not known but it is well established that the hydrogen ion concentration of the blood increases rather suddenly during the induction of anesthesia and then rises gradually during its maintenance. Although the ether and chloroform may exert a direct glycogenolytic influence upon liver glycogen, acidosis may play an important rôle in the production of this effect. A remarkable rise in blood sugar may occur during anesthesia. The degree of hyperglycemia is dependent upon the amount of glycogen in the liver, the quantity of anesthetic administered and upon the adequacy of the mechanism of carbohydrate utilization.

In the absence of any other factor which tends to produce hyperglycemia, such as hyperthyroidism or diabetes mellitus, the blood sugar has been found to rise 7-8 mg. per 100 cc per ounce of ether administered. These figures may be greatly increased if the anesthetic is not skillfully administered, by the introduction of complicating factors such as excessive muscular effort, excitement and asphyxia. In diabetes mellitus the degree of hyperglycemia is much more marked as the excessive amount of glucose entering the blood stream cannot be stored or utilized adequately by the tissues. The duration of anesthesia hyperglycemia, usually four to twelve hours after operation, depends upon the quantity of anesthetic used and the duration of the

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importance from the standpoint of therapy. Acidosis due to other causes, as fever, nephritis and dehydration, may result in an increase in the level of blood sugar. The rise in such cases is not marked, but, occurring in association with other factors, its influence may be significant.

Hepatic Disease. Although diminished glucose tolerance (p. 41) and a normal or slightly subnormal fasting blood sugar level (p. 34) occur much more commonly than fasting hyperglycemia in patients with hepatocellular damage, the latter is observed occasionally. Fasting values as high as 185 mg. per 100 cc. have been reported in patients with acute hepatitis in the absence of true diabetes mellitus. This is apparently the result of excessive glycogenolysis. In some cases, hyperglycemia in patients with hepatic or biliary tract disease may be due to associated pancreatitis, but this in itself seldom causes hyperglycemia. Fasting hyperglycemia, often marked, is a common but not invariable feature of hemochromatosis (p. 43), being due fundamentally to involvement of the islands of Langerhans in the fibrotic and degenerative process in the pancreas.

Miscellaneous. Strenuous muscular exercise causes liberation of glucose from the liver with resulting hyperglycemia. It must be realized that, as in all conditions acting through the agency of increased hepatic glycogenolysis, hyperglycemia can only be produced if the reserve supply of liver glycogen is abundant. When this is depleted, the blood sugar concentration decreases and, eventually, hypoglycemia may result. This fact is perhaps responsible for the contradictory reports of the blood sugar level in eclampsia and other convulsive states. Similarly, strenuous muscular exertion, in a normal individual, is at first associated with an increase and later, if continued, with a decrease in blood sugar, the source of supply having been virtually exhausted.

Fasting hyperglycemia may occur in many acute and chronic infections although usually the fasting blood sugar is within normal limits and the tolerance for glucose is lowered (p. 43). This is believed to be due to increased and prolonged hepatic glycogenolysis and gluconeogenesis, which may seriously aggravate a condition of latent or frank diabetes mellitus. A mild degree of hyperglycemia has been observed in about 5 to 40 per cent of cases of essential hypertension and occasionally in nephritic hypertension. Its incidence in the former condition is highest in patients with obesity, a condition that is frequently accompanied by a lowered tolerance for glucose (p. 44). Such patients (obesity and hypertension) may in reality be potential diabetics.

state of anesthesia. The rise appears to be more pronounced in laparotomies than in extra-abdominal operations but is otherwise independent of the nature or severity of the operative procedure. Values as high as 400 mg. per 100 cc. have been observed in individuals with normal carbohydrate metabolism.

If the quantity of glycogen stored in the liver is deficient, as in severe hepatocellular damage, the blood sugar cannot rise as in normal individuals and there is great danger of depletion of the hepatic glycogen reserve with consequent serious damage to the liver by the ether or chloroform which have a profound toxic effect upon the liver parenchyma. It has also been found that practically all of the barbituric acid series of hypnotics cause a rise in blood sugar and a simultaneous fall in the glycogen content of the liver. The effects of these various anesthetic agents may be attributable in part to asphyxia, to a direct action of the anesthetic on the liver or to liberation of epinephrine from the adrenal glands.

Anoxemia, whether due to mechanical causes or occurring in carbon monoxide poisoning or heart failure or during anesthesia, particularly with nitrous oxide and ethylene, is accompanied by an increase in blood sugar. The two anesthetic agents mentioned do not, in the absence of asphyxia, have any appreciable effect upon the blood sugar concentration. Anoxemia produces this effect probably by increasing mobilization of hepatic glycogen. The blood sugar may rise 20-40 mg. per 100 cc. after the administration of morphine, the mechanism probably being similar to that involved in asphyxia. Transient hyperglycemia and glycosuria have been reported in patients with acute coronary artery occlusion. The mechanism operating here may be due to sudden circulatory inefficiency.

Acidosis. Hepatic glycogenolysis apparently tends to be increased in the presence of acidosis. This may be due to the production in the liver of a more favorable hydrogen-ion concentration for the enzyme reactions upon which this phenomena depends.

This factor is of particular importance in diabetes mellitus because it aggravates a pre-existing hyperglycemic mechanism, in this case hypoinsulinism. In the evaluation of the significance of the fasting blood sugar concentration in this disorder it is important to attempt to determine what proportion of the elevation is dependent upon the complicating state of acidosis (ketosis). It is perhaps partly because of the hyperglycemic tendency in acidosis that such patients are relatively resistant to insulin; the recognition of this fact is therefore of great

importance from the standpoint of therapy. Acidosis due to other causes, as fever, nephritis and dehydration, may result in an increase in the level of blood sugar. The rise in such cases is not marked, but, occurring in association with other factors, its influence may be significant.

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Anoxemia, whether due to mechanical causes or occurring in carbon monoxide poisoning or heart failure or during anesthesia, particularly with nitrous oxide and ethylene, is accompanied by an increase in blood sugar. The two anesthetic agents mentioned do not, in the absence of asphyxia, have any appreciable effect upon the blood sugar concentration. Anoxemia produces this effect probably by increasing mobilization of hepatic glycogen. The blood sugar may rise 20-40 mg. per 100 cc. after the administration of morphine, the mechanism probably being similar to that involved in asphyxia. Transient hyperglycemia and glycosuria have been reported in patients with acute coronary artery occlusion. The mechanism operating here may be due to sudden circulatory inefficiency.

Acidosis. Hepatic glycogenolysis apparently tends to be increased in the presence of acidosis. This may be due to the production in the liver of a more favorable hydrogen-ion concentration for the enzyme reactions upon which this phenomena depends.

This factor is of particular importance in diabetes mellitus because it aggravates a pre-existing hyperglycemic mechanism, in this case hypoinsulinism. In the evaluation of the significance of the fasting blood sugar concentration in this disorder it is important to attempt to determine what proportion of the elevation is dependent upon the complicating state of acidosis (ketosis). It is perhaps partly because of the hyperglycemic tendency in acidosis that such patients are relatively resistant to insulin; the recognition of this fact is therefore of great

Apart from this form of induced hyperinsulinism, a similar mechanism may be brought into play by other means. In normal individuals, two and one-half to three and one-half hours after the administration of carbohydrate or epinephrine there is a period of mild hypoglycemia (50-70 mg. per 100 cc.) following the primary rise in blood sugar. It has been assumed that the mechanism of glycogen storage, stimulated by the rise in blood sugar, acquires a momentum which carries the process beyond the period of increased glucose supply. However, Soskin⁸¹⁻⁸⁶ demonstrated that the hypoglycemic reaction following cessation of prolonged sugar administration to animals does not depend upon increased mobilization of insulin from the pancreas, since it occurred in depancreatized animals receiving a constant supply of insulin. He believes that the fall in blood sugar which follows the primary rise accompanying the absorption of ingested sugar is due to a decrease in the supply of glucose by the liver to the blood, in response to the influx of exogenous sugar. He further believes that the period of hypoglycemia corresponds to the time which elapses before the liver is able to accelerate its rate of supply of blood sugar to a point sufficient to maintain the original normal blood sugar level.

Spontaneous hyperinsulinism may occur as a result of hyperplasia, adenoma and, rarely, carcinoma of the islands of Langerhans of the pancreas. The fasting blood sugar may be as low as 20-50 mg. per 100 cc., but is not necessarily consistently subnormal. In such cases, hypoglycemia may be induced or accentuated by (a) moderate exercise after a prolonged fasting period or (b) performing an oral or, preferably, an intravenous glucose tolerance test, the hypoglycemic phase of the blood sugar curve being characteristically exaggerated and prolonged (p. 44). In establishing the diagnosis, significant hypoglycemia must be shown to be present at the time of occurrence of the characteristic subjective and objective manifestations, which must also be largely relieved promptly by intravenous injection of glucose. It must be borne in mind, however, that the occurrence or severity of symptoms bears no constant relation, even in the same subject, to the actual level of the blood sugar. The rapidity as well as the extent of the fall may be important in this connection. In cases of latent or mild hyperinsulinism, the condition may be evidenced only by an exaggerated response to insulin administration (insulin tolerance test, p. 49).

Hypoglycemia due to hyperinsulinism occurs at times in the newborn of mothers with uncontrolled diabetes. In some fatal cases no morphologic abnormalities have been observed in the pancreas, whereas in others there has been hyperplasia or

Moderate elevations of blood sugar may occur in acute and chronic pancreatitis, but this is by no means a constant finding. An increase occurs as a result of smoking, due probably to the epinephrine-stimulating effect of nicotine. It may occur also after administration of caffeine, quinine, pituitrin and benzedrine (amphetamine), and has been reported in methyl salicylate poisoning.

FASTING HYPOGLYCEMIA

Postabsorptive hypoglycemia is encountered much less frequently than hyperglycemia. However, with the development of more specific knowledge regarding the influence of the liver and pancreas upon carbohydrate metabolism, hypoglycemia has achieved a clinical significance which was formerly lacking. The factors which tend to lower blood sugar are the opposite of those which have been dealt with in the discussion of hyperglycemia: (a) decreased rate of entrance of glucose into the blood (decreased hepatic glycogenolysis and gluconeogenesis); (b) increased rate of removal of glucose from the blood (increased storage or utilization in the extrahepatic tissues). The latter seldom produces fasting hypoglycemia by itself, except under unusual conditions of severe and prolonged muscular activity (marathon runners), but a tendency toward hypoglycemia due to other causes may be aggravated by exercise.

Hyperinsulinism.^{22,33,35,95} Following injection of insulin the blood sugar concentration falls, usually reaching a minimum in two to four hours, the degree of reduction and duration of hypoglycemia depending upon the amount of insulin administered. After protamine zinc insulin, the blood sugar usually reaches a minimum in twelve to sixteen hours, the effect lasting for about twenty-four hours. Symptoms of hypoglycemia usually occur when the blood sugar level reaches 45 mg. per 100 cc. (Folin-Wu) although some patients apparently have an extraordinary tolerance for low levels of blood sugar. In a case of diabetes reported by Peters and Rabinowitch the blood sugar ranged from 23-31 mg. per 100 cc. over a period of six hours without symptomatic manifestations. Since values below 25 mg. (Folin-Wu) probably represent nonglucose reducing substances, sugar was practically absent from the blood stream in this case. It is now well established that the true blood sugar is practically zero at the time of occurrence of hypoglycemic convulsions in rabbits. It is obvious that hypoglycemia of any degree can be induced by the injection of insulin, the responsiveness of different individuals varying within rather wide limits.

pleting this reserve, may result in serious consequences (postanesthetic hypoglycemia). Excessive, continued muscular exertion may be associated with hypoglycemia following the primary period of hyperglycemia, due likewise to secondary exhaustion of hepatic glycogen; this is also true of convulsive disorders such as occur in strychnine poisoning, eclampsia, uremia and tetanus.

Fasting hypoglycemia, usually mild but occasionally below 30 mg. per 100 cc., may occur in glycogen storage disease (von Gierke's disease),⁹¹ usually in association with ketonuria and an increase in blood glycogen (over 15 mg. per 100 cc.). This condition is characterized by an excessive accumulation of glycogen in the liver and other organs due to, or at least associated with, an inability to mobilize this substance, as evidenced by a diminished blood sugar response to epinephrine (p. 50) and increased sensitivity to insulin (p. 49). The tendency toward fasting hypoglycemia is believed due to the abnormal stability of the hepatic glycogen stores.

Reference has been made (p. 25) to the occasional occurrence of hypoglycemia after administration of insulin to patients with hemochromatosis. The situation may be analogous to that of the hepatectomized-depancreatized dog, in which the blood sugar rises higher after glucose than in the depancreatized dog and hypoglycemic manifestations develop more rapidly than in hepatectomized animals. This phenomenon does not always occur, certain patients with hemochromatosis exhibiting an extreme degree of insulin-resistance.

Adrenal Cortical Insufficiency. In Addison's disease, usually associated with atrophy or tuberculosis of the adrenal glands, the blood sugar is frequently low normal and occasionally subnormal. The average value in a large series of collected cases was 75 mg. per 100 cc. In a few instances figures of 30-40 mg. have been reported, usually in fatal cases shortly before death. This hypoglycemic tendency is due to diminished gluconeogenesis and perhaps also to diminished absorption of glucose from the intestine, resulting from adrenal cortical insufficiency. The defect in carbohydrate metabolism may be demonstrated most readily by performing an intravenous glucose tolerance test, the characteristic finding being exaggeration and prolongation of the hypoglycemic phase of the blood sugar curve (p. 46). Insulin sensitivity is increased (p. 49).

Hypoglycemia (0-30 mg.) occurring as a terminal event in extensive burns, diphtheria and scarlet fever (adrenal apoplexy or Waterhouse-Friderichsen syndrome), has been attributed to suprarenal failure. Longcope reported a case of scleroderma

hypertrophy of the islands of Langerhans. It is believed that during intra-uterine life prolonged exposure of the fetus to an abnormally high blood sugar concentration results in stimulation of excessive insulin secretion, with or without consequent hyperplasia of the islets. Following delivery, this increased insulin production often continues and exceeds the requirement of the infant, and hypoglycemia develops, frequently with serious consequences unless it is anticipated and treated promptly and adequately.

Hepatic Disease.^{15a, 70a, 93} Since the demonstration, by Mann and Magath, of the essential part played by the liver in the maintenance of the normal blood sugar level, several cases of hepatogenic hypoglycemia have been reported. The liver is endowed with such an extensive functional reserve capacity and with such remarkable regenerative powers that its glycogenic function is seriously impaired only in the late stages of chronic diseases such as cirrhosis. Blood sugar values of 50-60 mg. per 100 cc. may be observed rarely in the terminal stages of cirrhotic processes, particularly in obstructive biliary cirrhosis but at times also in the portal type. Hypoglycemia may be a terminal event after operations for biliary tract disease under general anesthesia which may exhaust a glycogen reserve already depleted by associated hepatic disease (hepatitis).

It is in the acute, rapidly progressive and extensive forms of hepatic disease that hypoglycemia is most commonly observed. Values as low as 25-40 mg. per 100 cc. have been reported in cases of phosphorus, benzol, chloroform and carbon tetrachloride poisoning and following administration of arsphenamine, synthalin and sulfonamides, usually, however, in terminal stages. Severe acute infections, such as diphtheria and scarlet fever, may produce similar grades of hypoglycemia, perhaps in the same manner, although suprarenal insufficiency may be a factor in such cases. Extremely low values have been observed in acute yellow atrophy or acute diffuse necrosis of the liver (15-50 mg.), acute and chronic infectious hepatitis and cholangiolitis, and in fatty liver. Marked hypoglycemia (25 mg.) has been reported in association with primary liver cell carcinoma replacing 70-80 per cent of the liver substance; at the time of death the blood sugar had fallen to 13 mg. per 100 cc. It is important to realize that other disturbances of function may not be demonstrable in spite of the fact that hepatic damage may be so extensive that the glycogen content of the liver is extremely low. The consideration of the state of the glycogen reserve is of the utmost importance in surgical disorders of the biliary tract since the administration of ether in such cases, by de-

Miscellaneous. Mild hypoglycemia has been reported in cases of status thymicolymphaticus, progressive muscular atrophy, pregnancy, lactation, renal glycosuria, and in anorexia nervosa and other conditions accompanied by severe grades of undernutrition.

ABNORMAL ALIMENTARY RESPONSE

EXAGGERATED RESPONSE—DIMINISHED GLUCOSE TOLERANCE

By diminished glucose tolerance is meant inability of the organism to handle ingested glucose as efficiently as a normal organism; it indicates inefficiency of one or more of several factors involved in the metabolism of carbohydrate:

(1) Inadequate glycogen storage capacity of the liver, so that glucose reaching the liver in the portal blood is not adequately removed, and, as a result, enters the systemic circulation in abnormal amounts. The chief clinical conditions in which this factor plays an important part are those in which there is extensive and rapidly progressive hepatic disease.

(2) Increased hepatic glycogenolysis and gluconeogenesis. In this group are included hyperthyroidism, hyperadrenalinism, acidosis and toxemia due to acute infections and adrenal cortical hyperfunction. These phenomena probably contribute also to the decreased glucose tolerance of diabetes mellitus. In the past, the generally accepted view has been that infection or toxemia affects the pancreas in such a way as to diminish insulin production or that they interfere with the action of insulin, whether of endogenous or exogenous origin. More recent studies have led to the hypothesis that the diminished glucose tolerance and relatively high insulin resistance associated with toxemia and infection are dependent, in part at least, upon acceleration of hepatic glycogenolysis. Some believe this to be due to increased epinephrine secretion, but it may be due to interference with the mechanism whereby the liver normally diminishes its supply of glucose to the blood in response to an influx of exogenous sugar (p. 9). A similar mechanism may also operate in the presence of hepatic functional impairment associated with various types of biliary tract disorder and liver disease included in the preceding group.

(3) Decreased tissue utilization. If the ability of the extra-hepatic tissues to form glycogen and to utilize glucose is diminished, an excess remains in the blood. The most important clinical condition in this group is diabetes mellitus (hypoinsulinism).

(4) Increased rate of absorption. An abnormally high rise in the blood sugar level may result from marked increase in the

with a blood sugar of 41 mg. per 100 cc. in which, at autopsy, one suprarenal gland was found to be atrophied.

Anterior Pituitary Insufficiency. The disturbance in carbohydrate metabolism is similar to that in adrenal cortical insufficiency. It is due in large measure to the same mechanism, being accompanied by atrophy of the adrenal cortex resulting from absence of the adrenocorticotrophic hormone. In addition, there is atrophy also of the thyroid (absence of thyrotrophic hormone), which further increases the tendency toward hypoglycemia and increases the glucose tolerance and the insulin sensitivity (pp. 47, 49). Spontaneous hypoglycemia may occur, with convulsions and coma.⁶¹ Fasting blood sugar values of 60 mg. per 100 cc. or lower have been reported in 43 per cent of forty-two verified cases of Simmonds' disease (pituitary cachexia) (range, 24-114 mg. per 100 cc.), in 14 per cent of ninety-six unverified but clinically typical cases (range, 36-138 mg.) and in 7 per cent of eight-three suggestive cases (range 35-153 mg.).²² A similar finding was obtained in 21 per cent of fourteen cases of anorexia nervosa (range, 26-134 mg. per 100 cc.), rendering the occurrence of spontaneous hypoglycemia of limited value in the differential diagnosis of these conditions.

A blood sugar of practically zero has been observed in a patient with diabetes mellitus following infarction of the hypophysis with destruction of the anterior lobe.⁴⁴ Hypoglycemia has resulted from extensive destruction of the anterior hypophysis by a chromophobe adenoma.

Hypothyroidism. In myxedema and cretinism the blood sugar concentration may be low, values of 50-60 mg. per 100 cc. (Folin-Wu) having been reported. Values of 70-80 mg. are commonly observed. A drop in blood sugar (60 mg. per 100 cc.) may occur at times immediately following thyroidectomy in individuals who have not received adequate amounts of carbohydrate before operation.

Nervous System Disorders. Fasting hypoglycemia, usually of mild degree, has been observed occasionally in vagotonia, "neurocirculatory asthenia," various psychoses, paresis, subarachnoid hemorrhage, the postencephalitic syndrome and certain brain stem (hypothalamic) lesions.^{67, 69, 70, 95} Increased glucose tolerance (p. 48) occurs much more commonly than actual hypoglycemia in such cases. The mechanism underlying the abnormality in carbohydrate metabolism is believed to be disturbed nervous control of hepatic glycogenolysis, a similar condition having been produced experimentally by injury to the paraventricular nucleus in rabbits⁶¹ and the hypothalamic region in cats and dogs.^{6, 17}

Miscellaneous. Mild hypoglycemia has been reported in cases of status thymicolymphaticus, progressive muscular atrophy, pregnancy, lactation, renal glycosuria, and in anorexia nervosa and other conditions accompanied by severe grades of undernutrition.

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(4) Increased rate of absorption. An abnormally high rise in the blood sugar level may result from marked increase in the

rate of absorption of glucose from the intestine, believed by some to occur in hyperthyroidism.³

The chief features of the blood sugar curve following the ingestion of glucose (Table 1, p. 40) which characterize a diminished glucose tolerance, are:

(a) An abnormally high rise in the venous blood sugar concentration (above 160 mg. per cent).

(b) The maximum concentration is reached later than in the normal individual (after one hour).

(c) The period of hyperglycemia is longer than normal.

(d) The return to the postabsorptive level is delayed (more than two hours).

In conditions in which the dominant factors are abnormally rapid absorption (e.g., hyperthyroidism), inadequate storage in the liver (e.g., hepatic disease) or excessive hepatic glycogenolysis (e.g., hyperadrenalinism, acidosis, toxemia), the most marked abnormality occurs in the first phase of the glucose tolerance curve: i.e., there is an excessively high rise in the blood sugar, but the return to the control level is not as long delayed as in conditions in which there is believed to be impaired tissue storage and utilization of glucose. The latter condition is observed characteristically in diabetes mellitus, the blood sugar curve being abnormally high and markedly prolonged.

Diabetes Mellitus. Diminished glucose tolerance is the most characteristic metabolic feature of this condition, occurring regardless of the postabsorptive blood sugar concentration. It is probably dependent in part upon disturbance of the homeostatic mechanism in the liver (p. 5), resulting in continuation of hepatic glycogenolysis and gluconeogenesis at abnormally high blood sugar levels, and also upon diminished capacity of the tissues for storing and utilizing glucose at normal blood sugar levels.

The sugar tolerance curve in diabetes mellitus has the following characteristics:

(a) Fasting hyperglycemia is present in all but very mild cases.

(b) A gradual rise to an excessively high level (above 160 mg. per 100 cc.), the degree of elevation being approximately proportional to the severity of the condition.

(c) The maximum concentration is reached after a variable interval following the ingestion of glucose but practically always more than one hour. In general, the greater the rise, the longer is the time elapsing before the highest level is attained. In severe cases the peak may not be reached for three or more hours.

(d) The most characteristic feature is the delayed return to the postabsorptive level. A failure to return to normal at the end of three hours is usually indicative of diabetes mellitus. After reaching a maximum, the blood sugar concentration remains at a high level for a variable period, decreasing slowly to the fasting level. The greater the elevation the slower is the rate of decrease, both paralleling the severity of the condition. Characteristically the curve is of the high plateau rather than the peaked type.

(e) The arterial-venous difference is often diminished.

Other phenomena may occur which suggest impaired glucose utilization. The respiratory quotient may remain at the post-absorptive level (p. 16) instead of exhibiting the normal increase which attends active carbohydrate storage and combustion. However, this may also be interpreted as due to increased gluconeogenesis from protein and fat.⁸² The serum phosphate concentration characteristically remains unchanged instead of decreasing during the period of hyperglycemia; in some cases it may increase slightly.

The glucose tolerance test is of particular diagnostic value in cases in which the fasting blood sugar is normal or only slightly elevated. The intravenous test is practically never indicated in cases in which diabetes mellitus is suspected and, since interest is centered chiefly in the first phase of the curve, the test may usually be terminated at the end of two or three hours for all practical purposes in such cases.

Abnormal One-hour, Two-dose Test

Exton found that in the one-hour, two-dose test in mild diabetes, the first part of the curve is similar in trend to that obtained in the ordinary one-dose glucose tolerance test. After the second dose of glucose, however, the blood sugar rises sharply, this feature distinguishing diabetes from all other conditions, according to this author. In more severe diabetes the responses to the one-hour, two-dose test are in general the same as the responses in mild diabetes, except that the sugar values are higher in all the blood and urine samples in the more severe cases. According to Exton, the criteria for determining diabetes in the one-hour, two-dose test are (1) a more or less steep rise of not less than 10 mg. of blood sugar in the sixty-minute sample following the second dose of glucose and (2) the relation of blood and urine sugar values to the severity of the disease. The criteria of alimentary glycosuria are (1) a sugar-free urine after fasting, with sugar in the final urine specimen, and (2) blood sugars that follow the normal curve even when the level is higher than

normal. The criteria for renal glycosuria are (1) blood sugars which follow the normal course, or in any event which never reach the diabetic level, and (2) sugar in both urine specimens.

According to Exton, this procedure is more valuable than the ordinary glucose tolerance test in that it frequently yields normal findings in nondiabetic subjects in whom abnormal

TABLE I
DECREASED GLUCOSE TOLERANCE

Condition		Fast- ing	Time after glucose			
			30 min.	60 min.	90 min.	120 min.
Normal (p. 8)	Venous sugar. . .	90	125	130	100	85
	Arterial sugar.	90	135	155	105	90
	A-V difference.	0	+10	+25	+5	+5
	Serum P.	4.0	3.4	2.9	2.5	2.8
	R.Q.	0.82	0.84	0.88	0.90	0.95
Decreased hepatic glycogenesis (p. 41)	Venous sugar.	60	140	170	130	90
	Arterial sugar.	60	150	190	145	100
	A-V difference. .	0	+10	+20	+15	+10
	Serum P.	4.0	3.6	3.0	2.8	3.1
	R.Q.	0.82	0.84	0.86	0.89	0.92
Increased hepatic glycogenolysis (p. 42)	Venous sugar.	110	165	190	170	135
	Arterial sugar.	110	180	210	185	140
	A-V difference.	0	+15	+20	+15	+5
	Serum P.	4.0	3.5	3.1	2.6	2.6
	R.Q.	0.86	0.88	0.90	0.95	0.96
Diabetes mellitus (p. 38)	Venous sugar.	180	260	310	340	350
	Arterial sugar.	180	265	318	350	355
	A-V difference .	0	+5	+8	+10	+5
	Serum P.	4.0	3.9	4.0	4.0	4.0
	R.Q.	0.76	0.76	0.77	0.78	0.77
Hyperpituitarism (p. 42)	Venous sugar . . .	110	150	170	160	130
	Arterial sugar	110	155	180	165	135
	A-V difference. .	0	+5	+10	+5	+5
	Serum P. . . .	4.0	3.8	3.8	3.9	4.0
	R.Q.	0.79	0.80	0.81	0.82	0.82

or questionable results are obtained by the latter method. In our experience with hospital patients, suffering from a variety of disorders, this procedure has not proven superior to the older test. Distinctly abnormal curves were obtained in many patients in whom no evidence of diabetes could be demonstrated and in whom essentially normal curves were obtained by the single

dose procedure. Similar findings were reported by Gould, who states that whereas all of his diabetic patients satisfy the criteria of Exton, 37 per cent of his nondiabetic patients yielded findings which would be regarded as indicative of diabetes by these criteria. He believes that diabetes mellitus may be correctly diagnosed if at least two of the following three conditions are encountered: (1) A fasting blood sugar above the upper limit of normal. (2) A half-hour blood sugar which exceeds the fasting level by 50 mg. or more. (3) A sixty-minute blood sugar which exceeds the half-hour level by 30 mg. or more. According to Matthews,⁵⁹ the most reliable criterion for the diagnosis of diabetes with this procedure is a one-hour blood sugar above 160 mg. per 100 cc. We concur in this opinion.

Hepatic and Biliary Tract Disease. As stated by Friedenson, "If the sugar which enters the blood after the ingestion of glucose is removed both by the tissues and by the liver, one should expect very definite abnormalities in the curve of alimentary glycemia of persons who have liver disease. Because such individuals partially or totally lack one of the mechanisms that reduce hyperglycemia, hepatic glycogen formation, the curve would presumably be excessively high or prolonged. On the other hand, the power of the tissues to remove glucose remaining intact, the arterial-venous difference should manifest itself in the normal manner." Unfortunately, however, from a diagnostic standpoint, this theoretical observation is not always borne out in fact. A normal alimentary response may be obtained in the presence of advanced hepatic disease. This is particularly true of chronic disorders such as various types of cirrhosis, passive congestion, carcinoma, and lues of the liver. This is in all probability due to the great functional reserve and remarkable regenerative power of that organ. In the acute diffuse forms of hepatic disease mentioned below, significant alterations in the blood sugar tolerance curve occur much more frequently. Such abnormalities have been described in acute and subacute hepatic necrosis, various forms of hepatitis, fatty liver, catarrhal jaundice, obstructive jaundice, acute alcoholism, portal cirrhosis and poisoning with phosphorus, chloroform, carbon tetrachloride, sulfonamides and arsphenamines. An abnormally high rise, occasionally prolonged, with an exaggerated hypoglycemic phase, has been observed in glycogen storage disease (von Gierke's disease).

The type of curve obtained in typical cases of insufficiency of hepatic glycogenic function is characterized by these factors:

(a) Abnormally high rise in venous blood sugar concentration (above 160 mg.).

(b) The maximum concentration is attained at the end of three-fourths to one and one-half hours, and in most instances within one hour.

(c) The blood sugar level usually falls rather rapidly, returning to normal within two to three hours. Only in cases of extreme grades of hepatic insufficiency is hyperglycemia of long duration.

(d) The arterial-venous difference is usually normal or, in some instances, increased.

(e) The combination of a low normal or subnormal fasting blood sugar level with a tolerance curve having the characteristics described above is highly suggestive of diminished hepatic glycogenesis.

If the determinations of the respiratory quotient and serum phosphate are made at frequent intervals after the administration of glucose they will be found to change in an essentially normal manner; *i.e.*, the R.Q. rises and the serum phosphate falls during the period of increased glucose supply and utilization (see pp. 15, 16).

Hyperthyroidism. Decreased tolerance for glucose occurs in 50-80 per cent of patients with hyperthyroidism^{4, 16} but does not necessarily parallel the severity of the condition. It is probably due in part to an increased rate of absorption from the intestine and in part to increased hepatic glycogenolysis. The blood sugar curve is not as characteristically plateau in type as that in diabetes mellitus, usually rising to a high peak earlier and descending earlier than in the latter condition.

Hyperfunction of the Anterior Pituitary and Adrenal Cortex.

Cushing's syndrome, whether due to primary hyperfunction of the anterior pituitary (basophilic adenoma or hyperplasia) or primary adrenal cortical tumor or hyperplasia, is characteristically accompanied by a state of decreased glucose tolerance. This is not present in all cases in early stages of the condition, but develops eventually almost invariably. It is dependent largely upon excessive gluconeogenesis (pp. 21-23) and, in the case of pituitary hyperfunction, perhaps also upon effects exerted through the thyroid and islands of Langerhans (pp. 20, 21). The curve in mild cases may resemble that seen in hyperthyroidism, acute infections, "toxemia," etc., but in more severe cases it is usually of the characteristic diabetic type.

The glucose tolerance varies considerably in acromegaly, but is usually decreased at some phase of the development of the condition.^{19, 27} In advanced cases, with destruction of the remainder of the gland by the eosinophilic tumor, normal curves may be obtained. The variable findings may also be due to opposing influences in this connection of factors stimulating

gluconeogenesis from protein and of the factor or factors stimulating growth and thus tending to conserve protein.

Glucose tolerance is at times normal in the adrenogenital syndrome, characterized by precocious puberty with either isosexuality in children, masculinization in the female or feminization in the adult male. In some instances, however, a diabetic type of curve may be obtained. Patients with adrenal medullary hyperfunction (pheochromocytoma) exhibit decreased tolerance for glucose during, and at times between, the characteristic attacks of paroxysmal hypertension and hyperglycemia (p. 28).

Miscellaneous. Decreased tolerance for glucose is commonly observed during pregnancy, particularly in the later stages. It is seldom of extreme grade and is usually associated with no elevation of the fasting blood sugar level. It is believed to be due to the disturbance of endocrine function (increased thyroid and pituitary activity) which exists during that period.

Diminished tolerance to glucose is commonly observed in patients with severe anemia, including pernicious anemia. It appears to occur most constantly in cases in which the free HCl of the gastric juice is low or absent. Some observers have reported an improvement in glucose tolerance associated with or following administration of HCl. Diminished tolerance occurs commonly also in patients with essential hypertension.

Fasting hyperglycemia and diminished glucose tolerance are at times observed in patients with glomerulonephritis and nephrosclerosis, particularly in the presence of renal failure. There is no associated disturbance of glucose utilization, the respiratory quotient rising in a normal manner following the ingestion of glucose. As stated by Linder and his co-workers, the abnormality apparently lies in a retarded transfer of glucose from blood to tissues rather than in retarded combustion of glucose in the tissues.

Decreased glucose tolerance may occur in certain central nervous system disorders, including head injuries or other causes of increased intracranial pressure (brain tumor, abscess, hemorrhage), hypothalamic lesions, emotional instability and, at times, dementia precox and manic-depressive insanity.

It also occurs in about 75 per cent of cases of hemochromatosis (fibrosis and degeneration of the islands of Langerhans) and occasionally in acute and chronic pancreatitis, due to involvement of the islands of Langerhans and to associated hepatic and biliary tract disease.

Various infectious and toxic states often depress glucose tolerance. This is particularly true of pyogenic infections, espe-

cially of the skin (furunculosis, carbuncle), but diminished tolerance may also occur in such conditions as rheumatoid arthritis, diphtheria, scarlet fever, pneumonia, tuberculosis, and others. The influence of infections is perhaps exerted through the medium of the liver, *i.e.*, by decreased responsiveness of the mechanism of hepatic glycogenolysis and gluconeogenesis to an increase in the blood sugar concentration (p. 31).

Curves indicative of diminished tolerance may be obtained in subjects with simple obesity (improving after weight-reduction), essential hypertension, arteriosclerosis, and Paget's disease (osteitis deformans), in about 50 per cent of cases of advanced malignancy and certain other conditions accompanied by severe undernutrition, in infants with extreme dehydration and in subjects in whom the carbohydrate intake has been restricted prior to the performance of the test.

DECREASED RESPONSE—INCREASED GLUCOSE TOLERANCE

By increased glucose tolerance is meant increased ability of the organism to handle glucose. The alimentary blood sugar response may be affected in one or both of two ways: (a) the initial blood sugar rise (hyperglycemic phase) is abnormally low or, at times, entirely absent, and/or (b) the hypoglycemic phase is abnormally pronounced and prolonged. In the majority of cases the latter phenomenon is elicited most readily by means of the intravenous glucose tolerance test. Spontaneous hypoglycemia may or may not be present. Subjects with increased tolerance for glucose almost invariably also exhibit increased sensitivity to insulin.

Hyperinsulinism. [As indicated elsewhere (p. 33), this condition may occur as a result of simple hyperfunction, hyperplasia, adenoma or carcinoma of the islands of Langerhans of the pancreas. The metabolic phenomena may be considered the result of a constant and excessive supply of insulin instead of a periodic secretion in response to the requirements of the organism. The characteristic blood sugar curve following administration of glucose should theoretically resemble that which follows the simultaneous administration of glucose and a large dose or repeated doses of insulin.

(a) Fasting hypoglycemia.

(b) Little or no elevation of blood sugar following glucose ingestion, resulting in a flat type of curve.

(c) Exaggeration of the hypoglycemic phase.

(d) Decrease in serum phosphate.

(e) Increase in the respiratory quotient.

However, the glucose tolerance curves in many cases of hyperinsulinism do not conform exactly to this description. In cases reported by Wilder and Howland, the blood sugar concentration in each instance rose to excessive heights (283 and 260 mg. respectively). This observation, suggestive of diminished sugar tolerance, was interpreted by Wilder as due to saturation of the liver with glycogen (8.25 per cent). Numerous studies in such cases have shown that the glucose tolerance test of three or four hours, as ordinarily carried out, is usually of little value in establishing a diagnosis of hyperinsulinism.³⁴ Although the low blood sugar values in the fasting state are significant and

TABLE 2
INCREASED GLUCOSE TOLERANCE

Condition		Fast- ing	Time after glucose			
			30 min.	60 min.	90 min.	120 min.
Decreased hepatic glycogenolysis	Venous sugar	65	70	80	74	68
	Arterial sugar	65	73	90	80	75
	A-V difference	0	+ 3	+10	+ 6	+ 7
	Serum P	4 0	3 2	2 6	2 2	2 2
	R.Q.	0 82	0 85	0 89	0 94	0 96
Hyperinsulinism (p 44)	Venous sugar	50	55	58	54	48
	Arterial sugar	50	70	90	65	58
	A-V difference	0	+15	+32	+11	+10
	Serum P	3 8	3 0	2 2	1 6	1 4
	R.Q.	0 88	0 90	0 94	0 96	0 96
Hypopituitarism (p. 47)	Venous sugar	70	78	82	76	70
	Arterial sugar	70	90	120	110	95
	A-V difference	0	+12	+38	+34	+25
	Serum P	4 0	3 2	2 6	2 4	2 4
	R.Q.	0 82	0 86	0 90	0 95	0 96

should arouse suspicion, the normal or even excessive rise during the first hour and the subsequent fall to a relatively normal level within three hours may be misleading. However, if one continues to make observations of the blood sugar concentration over a period of six hours, much more characteristic findings are usually obtained. After reaching a normal level, the blood sugar continues to fall and may be extremely low at the end of five to six hours. This procedure should be carried out in all cases of suspected hyperinsulinism

Abnormal prolongation of the hypoglycemic phase of the curve is of much greater significance than is the degree of hypo-

glycemia. This phenomenon is usually elicited best by the intravenous glucose tolerance test. The shape of the curve is influenced considerably by the nature of the antecedent diet,¹⁵ a high carbohydrate intake for a few days before the test favoring the production of a flat type of curve and a previously low carbohydrate intake often causing a normal or supernormal initial hyperglycemic phase. The insulin tolerance test (p. 48) is almost invariably abnormal, indicating increased sensitivity to insulin. In some instances, removal of an adenoma of the pancreatic islet cells is followed by a temporary state of hypoinsulinism, with diminished glucose tolerance, due probably to suppressed function of the islands of Langerhans.

Adrenal Cortical Insufficiency. This condition is characteristically associated with (a) increased tolerance for glucose, (b) increased sensitivity to insulin and (c) a decreased glycemic response to epinephrine. Some or all of the following abnormalities of carbohydrate metabolism have been observed in a large proportion of cases of Addison's disease:^{27,30}

(1) Low-normal or subnormal fasting blood sugar concentration (p. 35).

(2) Flat type of oral glucose tolerance curve, *i.e.*, diminished glycemic response after ingestion of glucose. This is due largely if not entirely to a diminished rate of absorption of glucose from the intestine and is the only abnormality of carbohydrate metabolism that is corrected by administration of desoxycorticosterone acetate. Intravenous administration of glucose under standardized conditions (p. 8) is followed by a rise in blood sugar to an essentially normal level, *i.e.*, the hyperglycemic phase of the curve is essentially normal.

(3) Exaggeration of the hypoglycemic phase of the curve after either oral or, more strikingly, intravenous administration of glucose. The blood sugar falls to an abnormally low level at two to three hours and may remain at this level for several hours instead of returning promptly to the control level, as in normal subjects. During this period, severe subjective and objective manifestations of hypoglycemia often develop at a higher blood sugar concentration than in the case of normal subjects.

(4) Marked prolongation of the hypoglycemic response to insulin²⁷ (p. 48), *i.e.*, "hypoglycemia unresponsiveness."

(5) Slightly decreased glycemic response to a standard dose of epinephrine.

(6) Striking hypoglycemia (a) during fever or infections, (b) on a diet high in fat and low in carbohydrate and (c) following a twenty-four-hour fast.

(7) High standard R.Q. (p. 16), which increases more than normally after administration of glucose.

These defects in carbohydrate metabolism are due to (a) diminished absorption of glucose from the intestine and (b) decreased gluconeogenesis from protein and perhaps fat (p. 22). The ability to form glucose from lactic acid and pyruvate as well as from glycogenic amino acids is apparently impaired.^{27,90} The diminished absorption can be corrected by administration of an adequate amount of sodium chloride or desoxycorticosterone acetate. This, as well as the more fundamental defects in gluconeogenesis and glycogenesis, can be corrected by adequate amounts of adrenal cortical extract, 17-hydroxy-11 dehydrocorticosterone (compound *E* of Kendall) or corticosterone.

Because of the ease with which hypoglycemic manifestations are produced in subjects with adrenal cortical insufficiency, provision must be made for prompt intravenous administration of glucose when either glucose or insulin tolerance tests are performed.

Anterior Pituitary Hypofunction. Increased tolerance for glucose (flat blood sugar curve) was reported in 52 per cent of twenty-one verified cases of pituitary cachexia (Simmonds' disease) due to destructive lesions, such as atrophy, hemorrhage, chromophobe tumor, and cyst, normal curves being obtained in 38 per cent and diabetic curves in 10 per cent.²³ It is difficult in such cases to evaluate the influence of severe undernutrition in this connection, as evidenced by the occurrence of a flat type of curve in about 30 per cent of cases of anorexia nervosa. However, in Simmonds' disease, there is characteristically a tendency toward spontaneous hypoglycemia, with increased glucose tolerance, similar to that in Addison's disease, and increased sensitivity to insulin.

The glucose tolerance is not so frequently altered in other types of pituitary hypofunction, such as pituitary infantilism or dwarfism. Although the glucose tolerance may be increased,¹⁶ fasting hyperglycemia and decreased glucose tolerance have been observed in such cases.^{8,75}

Hypothyroidism. A flat type of glucose tolerance curve is commonly obtained in cretinism and myxedema. This is due in part to a diminished rate of absorption from the intestine, for the intravenous glucose tolerance test yields essentially normal findings in many such cases.

It is interesting in this connection to note that Gilligan found the fasting blood sugar concentration to be normal in non-diabetic patients with hypothyroidism induced by total thyroidectomy. However, the postoperative sugar values tended to

be slightly lower than those obtained before operation. Similarly, although the hyperglycemia produced by glucose ingestion was usually slightly less after total thyroidectomy than previously, the glucose tolerance curve was within normal limits in non-diabetic patients with induced hypothyroidism. It was concluded that carbohydrate metabolism is not significantly influenced [by hypothyroidism induced by total thyroidectomy except when a derangement of carbohydrate metabolism is evidenced prior to operation. Marked improvement in glucose tolerance was observed in diabetic patients following removal of the thyroid gland, and similar findings were obtained in a patient with hyperthyroidism who presented a diabetic type of curve before operation.

Miscellaneous. A flat type of glucose tolerance curve may be obtained in renal glycosuria, in hypothalamic lesions, in some, but not all cases of glycogen disease (p. 35), due apparently to increased stability of the hepatic glycogen, with diminished glycogenolysis, and in idiopathic steatorrhea (celiac disease, sprue), probably as a result of impaired absorption of glucose from the intestine. Curves of this nature may be produced by conditions associated with increased intestinal motility and abnormal gastric emptying, such as vitamin B deficiency, "gastrointestinal neuroses," achlorhydria, tuberculous enteritis and chronic ulcerative or mucous colitis. In such cases, a normal response is obtained with the intravenous glucose tolerance test. Curves indicative of increased tolerance have been reported in marasmus in infants and in anorexia nervosa and may be produced in normal subjects by excessive carbohydrate feeding prior to the performance of the test.

ABNORMAL-INSULIN TOLERANCE

The purpose of the insulin tolerance test is to determine (a) the sensitivity of the organism to insulin and (b) its responsiveness to insulin-induced hypoglycemia. The normal blood sugar response to insulin is discussed elsewhere (p. 8). Abnormalities may occur in one or both of two phases of the curve, *i.e.*, in the extent and duration of the hypoglycemia and in the subsequent rise toward the pre-injection level. There are two important types of abnormality:²⁷ (1) "Insulin resistance," characterized by a relatively slight (less than 50 per cent of the control level) or delayed (forty-five minutes or longer) fall in blood sugar; (2) "Hypoglycemia unresponsiveness," characterized by undue prolongation of the period of hypoglycemia (absence of or marked delay in the subsequent rise in blood sugar).

The antecedent diet should be controlled as in the case of the glucose tolerance test. Under normal conditions, the effect of the insulin balances that of the glucose, so that little or no significant change occurs in the blood sugar concentration. The chief purpose of this test is to determine, in a subject who has a diminished glucose tolerance, whether this is due to insulin deficiency, or to insulin resistance. In diabetes due to insulin deficiency, the glucose-insulin tolerance curve will be essentially normal. In diabetes due to resistance to insulin, this curve will approach the ordinary glucose tolerance curve, indicating relative ineffectiveness of the exogenous insulin. This type of response is encountered also in some instances of acromegaly and of Cushing's syndrome, of either primary pituitary or primary adrenal origin.

ABNORMAL EPINEPHRINE TOLERANCE TEST

Diminished glyccemic response to epinephrine (rise of less than 35 mg. per 100 cc. in forty to sixty minutes) may occur in conditions in which the hepatic glycogen stores are depleted (in the absence of other disturbances of carbohydrate metabolism), particularly in advanced grades of hepatocellular damage (acute and subacute hepatic necrosis, various forms of hepatitis, fatty liver, cirrhosis, and so on). It is also observed characteristically in patients with glycogen disease (von Gierke), in which condition the liver contains an excessive quantity of glycogen which appears to be highly resistant to glycogenolytic stimuli. A subnormal response to epinephrine also occurs commonly in conditions in which a state of "hypoglycemic unresponsiveness" is demonstrated by means of the insulin tolerance test, viz., hyperinsulinism, Addison's disease and pituitary cachexia.

ABNORMAL TOLERANCE FOR OTHER SUGARS

Abnormal Levulose (Fructose) Tolerance.^{33,74,85} The metabolism of levulose differs in several essential details from that of glucose.^{18,20} There can be no doubt that after its ingestion fructose may enter the blood in unaltered form. However, for clinical purposes, only the total blood sugar concentration is determined, no distinction being made between fructose and glucose. Although there is some evidence that fructose may be utilized without preliminary transformation to glucose or glycogen, it is probably largely removed from the blood and transformed into glycogen before contributing to the blood glucose or possibly before undergoing oxidation. The liver appears to be the chief if not the only site of transformation of

fructose to glycogen. Indeed, it appears that fructose is a better glycogen-former than glucose and that it can undergo this transformation in the absence of insulin. Because of the specific importance of the liver in the intermediary metabolism of this sugar, the determination of the tolerance of the organism for levulose has been suggested as a means of detecting impairment of hepatocellular function (p. 414).

From a practical viewpoint a normal tolerance for levulose is rarely observed when the glucose tolerance is diminished. Hence the performance of the levulose tolerance test is of no clinical value in the presence of conditions such as diabetes mellitus. Its chief sphere of usefulness has been in the estimation of hepatic functional efficiency in the absence of other disturbances of carbohydrate metabolism. Following the ingestion of 45 Gm. of levulose, (p. 414), diminished capacity of the liver for transforming levulose to glycogen is evidenced by the following phenomena:

(a) A rise in blood sugar of more than 35 mg. per cent, a concentration of 135 mg. or more being reached at some period during the performance of the test.

(b) A delayed return to the postabsorptive level (beyond two hours).

The demonstration of diminished levulose tolerance in the absence of evidence pointing to any disturbance of general carbohydrate metabolism is suggestive of impairment of liver function. However, negative results are commonly obtained in the presence of advanced hepatic disease especially if chronic in nature (cirrhosis, lues and malignancy of the liver, etc.). Positive results may be obtained in more acute forms of liver disease, such as acute yellow atrophy, toxic necrosis or hepatitis associated with chloroform, arsenic, phosphorus and carbon tetrachloride poisoning, acute catarrhal jaundice, etc. As a general rule this test is of little practical clinical value in the estimation of liver functional efficiency since in most instances other evidence of functional impairment is present long before positive results are obtained. Of still less value is the procedure advocated by Strauss, in which the occurrence of fructosuria following the ingestion of 100 Gm. of fructose is assumed to be indicative of liver damage. At least 10 per cent of normal individuals respond by eliminating some fructose in the urine and many patients with hepatic disease yield findings within the limits regarded as normal.

More satisfactory results have been reported when the analytical procedure is modified so that the blood fructose (levulose) alone is determined, rather than fructose plus glu-

eose.^{38,68} In the absence of hepatocellular damage, after ingestion of 50 Gm. of levulose, the blood levulose concentration, usually 0-8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour, and falls to 0-10 mg. at the end of two hours. Increases of 16-30 mg., with a delayed fall to the resting level, have been observed in patients with hepatocellular damage. Similar findings have been obtained at times in patients with arteriosclerosis.

Abnormal Galactose Tolerance. In view of the widespread employment of the galactose tolerance test as a measure of liver function, interest centers particularly upon the part played by the liver in the intermediary metabolism of this sugar. It has been found that when galactose is injected intravenously in the normal dog it disappears from the blood in about two hours, 10 to 30 per cent appearing in the urine. Removal of 50 to 70 per cent of the liver has little effect upon the tolerance for galactose. In the absence of the liver, 50 to 60 per cent of the amount injected is recovered in the urine.¹⁰ Similarly, increased excretion has been observed in the presence of acute degenerative lesions of the liver produced experimentally by carbon tetrachloride, chloroform, phosphorus and similar hepatotoxic agents.

The usefulness of this procedure as a measure of hepatocellular function and as a means of differentiating between obstructive and hepatocellular jaundice is discussed in detail elsewhere (p. 415). Suffice it to state here that, with the oral test, the excretion by a jaundiced patient of less than 3 Gm. of galactose in five hours suggests that the jaundice is not of hepatocellular origin, while the urinary excretion of more than 4-5 Gm. suggests that it is. With the intravenous test, the presence of more than 20 mg. of galactose per 100 cc. of blood at the end of seventy-five minutes, in a patient with jaundice, suggests that the latter is of hepatocellular origin, while less than 20 mg. per 100 cc. at this time points toward obstructive jaundice.⁷

Determination of the increase in blood galactose after ingestion of 40 Gm. of this sugar has been proposed as a means of studying thyroid function.³ It has been found that in hyperthyroidism the peak of blood galactose concentration ranges from 25 to 150 mg. per 100 cc., being above 40 mg. in the great majority of cases (normal maximum 15-35 mg. per 100 cc.) This abnormally high rise is attributed to increased rapidity of absorption of the sugar from the intestine in patients with hyperthyroidism. Essentially normal results are obtained in hyperthyroidism when the galactose is given intravenously,

indicating that the above findings are not due to abnormality of intermediary metabolism. This procedure may be of value in differentiating hyperthyroidism from other conditions accompanied by increase in the basal metabolic rate that may simulate it clinically, especially congestive heart failure of nonhyperthyroid origin.

Decreased tolerance for galactose has been observed in hyperpituitarism, certain pluriglandular disturbances involving gonadal dysfunction and occasionally during menstruation and after the menopause.⁷²

BLOOD GLYCOLYSIS

The study of the rate of disappearance of glucose from blood *in vitro* first acquired distinct clinical significance when Warburg observed that the carbohydrate metabolism of tumor tissue differed from that of normal cells in that the former possesses the property of causing an increased rate of glycolysis in a glucose-containing medium. When normal whole blood, prevented from clotting by defibrination or by the addition of anticoagulants such as heparin, oxalates and citrates, is incubated at 37° C. its sugar content decreases at a practically uniform rate for four hours and is usually virtually exhausted at the end of six hours. The concentration of reducing substances at the end of this time is 10–20 mg. per 100 cc., representing non-glucose reducing substances (Folin-Wu).

This glycolytic process appears to be normally dependent upon the activity of the erythrocytes, as it is almost completely inhibited by their removal or after hemolysis has been produced. The number of normal leukocytes has apparently little influence, the rate of glycolysis being unaltered in the presence of leukocytosis (polymorphonuclear) of varying degree.

In polycythemia vera (erythremia) glycolysis usually occurs with increased rapidity, being complete in two to three hours. This appears to be independent of the number of red blood cells as influenced by the administration of phenylhydrazine. In cases of relative polycythemia glycolysis is normal.

A similar increased rate of glycolysis occurs in chronic myelogenous leukemia (two to three hours) except in aleukemic stages. Some observers state that the rate is determined by the number and degree of immaturity of the leukocytes, but it may be that other factors are involved. Normal findings are usually obtained in chronic lymphatic leukemia.

When normal, defibrinated blood is incubated at 37° C., glycolysis occurs at a fairly constant rate, usually amounting to a loss of 13–16 mg. per 100 cc. per hour until there remains a

residual reducing substance of about 20 mg. per 100 cc. Several observers have shown that the rate of blood glyeolysis is diminished in pernicious anemia. It was pointed out by Goldhamer that this retardation is proportional to the red cell decrease, the average rate per million red blood cells being approximately the same as that of normal subjects.

Guest found that at the time of disappearance of sugar (six to eight hours) the inorganic phosphorus of the blood rose sharply and steadily, reaching a concentration of about 20-25 mg. per 100 cc. at about the fiftieth hour. This increase in inorganic phosphorus occurred at the expense of the organic acid-soluble phosphorus of the red cells, generally designated "ester phosphorus." The increase in inorganic phosphorus occurred very quickly in the hypoglycemic blood of insulinized animals.

There has been considerable controversy as to the relationship between the rate of glyeolysis and the level of blood sugar. Some investigators report decreased glyeolysis in diabetes mellitus and in hyperglycemia due to other causes. Others deny the truth of this assertion. Falcon-Lesses reports that the decrease in blood sugar is more rapid when the blood sugar concentration is high. If one considers the amount of decrease per unit of time rather than the percentage decrease it is usually found that the rate of glyeolysis is practically independent of the degree of glycemia. Insulin has no effect upon glyeolytic activity.

NORMAL URINE SUGAR

In the normal individual glucose is excreted by the renal glomeruli but, constituting one of the so-called "threshold bodies," it is largely reabsorbed into the blood stream through the tubular epithelium. A small amount, however, escaping this conservation process, is eliminated in the urine. The presence of a detectable quantity of copper-reducing substance or substances in normal urine has been recognized for many years. Benedict, Osterberg and Neuwirth found that 1.5 Gm. of such substances may be eliminated in twenty-four hours and that their excretion is increased following the ingestion of food. The term "glycuresis" was applied by them to this phenomenon in substitution for the more commonly employed and misleading term, "glycosuria." The proportion of these reducing substances represented by glucose has been variously estimated. Neuwirth found the total quantity of reducing substances to range between 0.61 and 1.38 Gm. daily, of which 0.13-0.49 Gm. were fermentable and 0.37-1.02 Gm. nonfermentable. Benedict believes that glucose constitutes usually not more than 25 per

cent of the normal urine sugar. Greenwald is of the opinion that the reducing substances excreted in normal urine are made up of poorly or non-assimilable carbohydrates and substances derived from the protein of the food and from endogenous sources. He states that the nature of the former depends on the diet (lactose from milk, pentose from fruits, caramelized sugar and dextrins) and that on ordinary diets at least 50 per cent of urine sugars originate from food protein or endogenous sources.

The importance of the appreciation of the presence of reducing substances in the urine of normal individuals is obvious. It is important, also, to distinguish between glycuressis, a physiologic, and glycosuria, usually a pathologic phenomenon. This distinction may be summarized according to the conception of Folin and Berglund, as follows: glycuressis follows every ordinary carbohydrate meal, the increase in reducing substances being independent of the amount of glucose in the blood and being due largely to the excretion of foreign unassimilable carbohydrates and carbohydrate decomposition products produced during the preparation of food. A portion of these reducing substances is represented by glucose. In a study of 700 normal individuals, Hassan found that the application of the phenylhydrazine test showed glucosazone to be present in 20-30 per cent after one to two hours, in 12-15 per cent after four to five hours and in 7 per cent after twelve hours. On the other hand, the output of glucose is less after the ingestion of 50 Gm. of pure glucose than following an ordinary mixed meal, and the administration of as much as 200 Gm. of glucose is not followed by glycosuria. After meals of bread, and particularly in concentrated urines, the ordinary reduction tests may yield positive results. The fermentation test is commonly used to distinguish between glucose and noncarbohydrate reducing substances. However, yeast sometimes fails to ferment sugar present in concentrations below 0.1 per cent and bacteria and other agents present in the yeast negate the results if differentiation between glucose and other sugars such as lactose and maltose is attempted. Sumner states that the urine of normal individuals contains reducing substances in concentrations varying from 0.05 to 0.15 per cent in terms of glucose; about 60 per cent of the reduction is due to sugar. Values of 0.25 per cent are to be considered with suspicion and 0.3 per cent as definitely pathologic, especially if the urine is not concentrated.

RENAL THRESHOLD

The concept of a threshold limit of renal "impermeability" to glucose has served as a convenient basis for the classification

of various forms of glycosuria. The renal threshold may be defined as that concentration of sugar in the blood which must be reached before an excessive quantity (above normal) of glucose is eliminated in the urine. This "threshold value" is generally assumed to be normally from 140 to 160 mg. per 100 cc. of whole blood and 170 mg. per 100 cc. of plasma.

There are two diametrically opposed schools of thought in this connection. The one affirms its belief in the existence of a renal threshold for glucose. Folin and Berglund who are among the proponents of this belief state, "Hyperglycemia definitely below the threshold does not normally produce the slightest leakage of glucose through the kidneys and normally not a trace of absorbed and circulating glucose is lost." Likewise, Joslin states, "The concept comprised in the term 'glucose threshold' is not only approximately true, but absolutely correct, however uncertain the exact figures given for the threshold may be." Benedict and Osterberg, on the other hand, say, "The more we have hunted for the elusive 'glucose threshold,' the more we feel that this is quite possibly wholly an artifact. We tend to adopt the view that the causes leading to glucose excretion by the kidney are usually the same as those leading to an increase in the blood sugar, but we question that the two latter phenomena need be always causally related." Furthermore, Folin and Berglund, while affirming their belief in the existence of a threshold for glucose and levulose, admit that there is apparently none for galactose and lactose, the elimination of which is independent of their concentration in the blood.

There can be no question that the term "renal threshold" is misleading from a physiologic standpoint. As stated by Peters,⁶⁸ the concept of renal threshold appears to be that of a barrier or dam. As long as the concentration of glucose in the blood plasma remains below the top of the dam it does not appear in the urine; when it rises above this level it passes over the dam and continues to escape in the urine until the concentration in the plasma has fallen below the critical level. As stated by this author, this concept is inconsistent with established facts. Glucose passes freely from the blood into the glomerular filtrate, its concentration in the latter being approximately the same as in blood plasma.⁷² Under normal conditions it undergoes practically complete reabsorption in the tubules, probably in the proximal convoluted tubules.^{72, 92} As stated by Peters,⁶⁸ when its concentration in the glomerular filtrate, and in the blood about the tubules, is unusually high, the tubular absorptive mechanism becomes inadequate, and some glucose escapes in the urine. The point at which this occurs is extremely variable, depending upon

physiologic conditions which are not well understood. Experimental evidence suggests that the total quantity of glucose that enters the tubules is of much greater significance in determining the occurrence of glycosuria than is the concentration of glucose in the blood. The normal human kidney is capable of reabsorbing a maximum of 250-350 and perhaps as much as 450 mg. of glucose per minute; similar values have been obtained in patients with diabetes.^{28,30,55,76,78,87} When the quantity of glucose that passes through the glomerular filter per minute exceeds these values, its reabsorption in the tubules is incomplete, regardless of the blood sugar level. The concept of a "renal threshold" for glucose is acceptable clinically if it is applied to this entire phenomenon of filtration and reabsorption, inasmuch as the quantity of glucose entering the tubules per minute is dependent upon the concentration of glucose in the blood plasma under normal conditions of glomerular function. From a practical standpoint, therefore, it is satisfactory to assume that tubular reabsorption of glucose becomes incomplete and glycosuria occurs when the blood sugar concentration rises to an excessively high level.

The concept of such a threshold is useful from a clinical standpoint. It must be recognized, however, that the "threshold value," if such exists, is extremely variable, varying not only in different individuals, but also in the same individual at different times. This may be due to one or more of several causes:

(1) The "permeability" of the kidney for sugar is dependent not only on the level of blood sugar at that moment but also upon the duration of an existing hyperglycemia.

(2) Blood and urine (bladder) removed at the same time do not represent simultaneous specimens, for the rate of urine formation varies as does the blood sugar concentration during and prior to the period of urine formation.

(3) The concentration of sugar in venous blood may not always be a true index of its concentration in the arterial blood supplying the kidney.

(4) The relationship between the level of sugar in the blood and its excretion in the urine varies with rising and with falling blood sugar values. It has been found that, following the administration of glucose, its elimination in the urine began when the blood sugar concentration was 150 mg. per cent and continued until it had dropped to 60 mg. According to Folin this is due to the fact that, prior to the excretion of the sugar, the holding capacity of the tissues, including the kidney, is exceeded, thus producing a local functional strain with the consequence that

the glycosuria, once begun, does not stop when the blood sugar has fallen to the threshold value or even lower.

If one admits the practical usefulness of the concept of a renal threshold for glucose, it must be realized that the threshold level possesses a wide individual variation and is capable of extreme variation under certain circumstances. Glycosuria has been observed in normal persons with a blood sugar concentration of 60 mg. per cent (Folin-Wu) and, as in a patient under ether anesthesia reported by Mackay, glycosuria may not occur in the presence of a blood sugar level of over 350 mg. per cent. The "renal threshold" is lowered in renal glycosuria, phlorhizin glycosuria and perhaps in normal pregnancy. It is frequently elevated in nephritis, nephrosclerosis and arteriosclerosis and in elderly patients with diabetes (nephrosclerosis?). In the last, blood sugar values as high as 425 mg. per 100 cc. have been reported without concomitant glycosuria.

The excretion of glucose in the urine is dependent upon three factors:⁴³ (a) the concentration of glucose in the arterial blood reaching the glomeruli, (b) the rate at which the glomeruli filter it out of the blood and (c) the rate of reabsorption of the filtered glucose by the tubular epithelium. Inasmuch as the concentration of glucose in the glomerular filtrate is the same as in the blood plasma, the quantity of glucose entering the tubules per minute is represented by the product of the glomerular filtration rate (*i.e.*, the inulin clearance, or urea clearance/0.6) and the concentration of glucose in the blood plasma. When the former is 125 cc. and the latter 100 mg. per 100 cc., 125 mg. of glucose will enter the tubules per minute. Since, under normal conditions, the tubules can reabsorb up to 250-350 mg. per minute, there will be no glycosuria. If the blood sugar is 300 mg. per 100 cc., 375 mg. will enter the tubules per minute, 25-125 mg. of which may escape reabsorption and pass into the urine. This is the condition in diabetes mellitus. However, if the glomerular filtration rate is simultaneously reduced to 80 cc. per minute, only 240 mg. of glucose will enter the tubules per minute and glycosuria may not occur despite a blood sugar level of 300 mg. per 100 cc. This might occur in diabetes mellitus complicated by nephrosclerosis or nephritis, unless there was at the same time sufficient tubular damage to diminish tubular reabsorption to the same extent as glomerular filtration.

With these facts in mind the statement may be made that sugar (glucose) is excreted in the urine when the level of blood sugar has risen above the normal "threshold level" for that individual. If the commonly accepted threshold values of 140 to 160 mg. are considered to be normal, individuals with glyco-

suria may be classed into two divisions: (1) with normal renal threshold and excessive hyperglycemia, and (2) with low renal threshold and normal blood sugar (renal glycosuria).

ABNORMAL URINE SUGAR

MELITURIA

The term "melituria" is properly employed to designate the presence, in the urine, of an abnormal amount of sugar. When the sugar is glucose the condition is termed "glycosuria," when levulose, "levulosuria," when pentose, "pentosuria," when lactose or galactose, "lactosuria" and "galactosuria," respectively. Since all meliturias are not glycosuria, the identification of the sugar present becomes a matter of considerable moment.

Tests for the Detection of Sugars

Metallic Oxide Reduction Tests. The most widely used routine method for the detection of sugar in the urine is one of the copper reduction tests of which the Benedict test is perhaps the most satisfactory. The property of reducing metallic oxides in alkaline solution (copper, bismuth, mercury), possessed by certain sugars, depends upon the presence of a free aldehyde or ketone group in their molecular structure. If Fehling's solution is employed, reduction may be caused by substances other than sugars if present in sufficient concentration. Among these are uric acid, nucleoprotein and conjugate glycuronates formed after the ingestion of antipyrine, menthol, phenol, camphor, chloral, etc. Creatinine may, by dissolving cupric oxide, mask slight degrees of reduction caused by small amounts of sugar. If chloroform is used as a preservative, a falsely positive result may be obtained.

Benedict's test is much more satisfactory than Fehling's. It yields positive results with glucose present in as low a concentration as 0.1 per cent. The Benedict reagent is furthermore less susceptible to reduction by uric acid and chloroform.

The bismuth reduction test (Nylander) is not commonly employed. It is believed to be capable of detecting smaller quantities of glucose than the Benedict reagent but albumin produces a black color similar to that produced by sugars and so, if present, must be removed before performing the test.

The following sugars are capable of reducing metallic oxides in alkaline solution. glucose, levulose, galactose, pentose, lactose and maltose.

Fermentation Test. The fact that certain sugars are fermentable by yeast has been the basis for the widespread use of

the fermentation test in the identification of urinary sugars. The statement is ordinarily made that glucose, levulose and galactose are fermentable by yeast and that maltose and sucrose are fermentable only after their inversion by the enzymes maltase and invertase present in the yeast. Lactose is said to be non-fermentable by ordinary bakers' yeast. One possible source of error has been indicated by Neuberg who demonstrated that yeast possesses the property of splitting off carbon dioxide from the carboxyl group of amino acids which are normally present in the urine.

Another important observation has been made by Castellani and Taylor who found that ordinary bakers' yeast is not pure and usually consists of one or two species of *saccharomyces* with a contaminating gram-positive bacillus. They showed that most cultures of so-called "pure yeast" ferment glucose, levulose, galactose, sucrose, maltose and, in many instances (15 per cent), lactose. Obviously, positive differentiation of urinary sugars on the basis of this test is impossible.

Castellani has elaborated a method of differentiating various sugars on the basis of fermentation by specific fungi and gas production by specific bacteria. For example, glucose alone is fermented by *Monilia balcanica*; glucose and levulose are fermented by *Monilia krusei*; *B. coli* forms gas with lactose, whereas *B. paratyphosus* does not. The reader is referred to the work of Castellani for further details. The combined use of reduction tests and gas production by specific fungi and bacteria is of great value in the positive identification of urinary sugars.

Phenylhydrazine Reaction. This reaction depends upon the formation of a crystalline osazone, the structure of which is typical, to a certain degree, for various sugars. Glucose and levulose form osazone crystals of identical structure. The identification of lactose by this test is not practicable for lactosazone crystals, although typical, are formed with difficulty in urine. At times the determination of the melting point of these crystals is utilized as a means of differentiating the sugars but it is not a procedure of practical clinical value.

Specific Rotation. The degree of rotation of polarized light, determined by means of a polariscope or polarizing saccharimeter may be employed as an aid in the identification of urinary sugars. This procedure is not frequently resorted to clinically. Furthermore, glucose and lactose cannot be differentiated by this method.

Other tests which are of value in the positive identification of urinary sugars will be dealt with in discussing the various types of melituria.

GLYCOSURIA

The term "glycosuria" signifies the excretion, in the urine, of abnormal amounts of glucose. Glucose may be identified in the urine on the basis of the following tests:

- (1) Positive reduction test.
- (2) Fermentation with bakers' yeast.
- (3) Typical glucosazone crystals with phenylhydrazine.
- (4) Gas production with *Monilia balcanica* (Castellani).
- (5) Specific rotation of polarized light.

As has been indicated, the properties of reducing power and fermentation by yeast are shared by many sugars and are therefore not specific for glucose. The following criteria may be established for the positive identification of glucose.

(a) Typical osazone crystals with phenylhydrazine in the presence of a negative Seliwanoff reaction (resorcinol-hydrochloric acid) to exclude levulose, or

(b) Gas production with *Monilia balcanica* (Castellani), or

(c) Specific rotation of polarized light ($+52.5^\circ$) in the absence of a positive mucic acid test to exclude lactose.

As has been previously indicated, the several forms of glycosuria may be conveniently classified clinically under two headings:

1. Glycosuria unassociated with hyperglycemia.
2. Glycosuria associated with hyperglycemia.

Nonhyperglycemic Glycosuria. Glucose may appear in the urine in the presence of a normal concentration of sugar in the blood. This condition may be produced experimentally by the administration of the glucoside phlorhizin. It is observed clinically in so-called "renal glycosuria" (renal diabetes), during pregnancy, and, as some believe, in the condition commonly termed "alimentary glycosuria."

Phlorhizin Glycosuria. The administration of phlorhizin, orally or, better, subcutaneously, is followed by glycosuria associated with a normal, and indeed, in many instances, a subnormal blood sugar concentration. The theoretical aspects of this interesting and physiologically important condition cannot be dwelt upon except as they serve to throw light upon the possible existence of forms of glycosuria observed clinically, dependent upon factors operating locally in the kidneys. There appears to be little doubt that, as von Mering concluded, phlorhizin glycosuria is in the true sense of the term a renal glycosuria. There is rather convincing experimental evidence which suggests that this substance produces its glycosuric effect by inhibiting reabsorption of glucose from the renal tubules. In

view of the possibility that phlorhizin diminishes the rate of absorption of glucose from the intestine by inhibiting phosphorylation (hexose-phosphate formation) (p. 1), it is interesting to speculate as to whether its action in inhibiting glucose absorption from the renal tubules may not be produced through the same mechanism.

Renal Glycosuria (Renal Diabetes). The frequency of incidence of this condition, also known as "benign" glycosuria and "diabetes innocens" is perhaps not as great as is commonly supposed. Joslin and his associates⁴⁸ found sixty-two cases among 18,000 cases of melituria and Fowler^{28a} seven in 4000 cases of melituria. However, it has been found much more frequently in army recruits (9-26 per cent of cases of glycosuria).^{9,67}

When rigid diagnostic criteria were established, Marble was able to find only sixteen cases in 9000 patients with glycosuria. He outlined the following standards for the diagnosis of true renal glycosuria:

(1) Fasting blood sugar within normal limits and a normal or supernormal flat glucose tolerance curve.

(2) Glucose should be present in every specimen of urine, whether voided in the fasting state or after a meal. The quantity of sugar in the urine should be largely independent of the diet, although it may vary somewhat depending on the amount of carbohydrate ingested.

(3) Carbohydrate utilization should be normal, as evidenced by determinations of respiratory quotient and serum inorganic phosphorus after glucose ingestion.

(4) There should be no disturbance of fat metabolism, ketosis being more likely to develop when the patient fasts than when he overeats.

(5) Moderate doses of insulin should have little or no effect upon the glycosuria.

This condition is believed by many to be hereditary and familial, and it seems likely that, once developed, it persists throughout the life of the individual. Folin and Berglund, in contrast to Marble, are of the opinion that it is of comparatively frequent occurrence, existing in 1-2 per cent of otherwise normal students whom they have studied. They believe, likewise, that the majority of instances of so-called "alimentary glycosuria" are, in reality, cases of renal glycosuria. The importance of its recognition depends upon its apparent harmlessness; so far as can be determined, it never results in diabetes mellitus or in any metabolic derangement whatsoever. As stated by Marble, practical danger in making the diagnosis of renal glycosuria lies in the confusion of this condition with potential or mild diabetes

mellitus. Care must be exercised also in the diagnosis of the glycosuria of pregnancy, which is not always of the benign type. He believes that the patient must be observed carefully over a period of at least three years before the diagnosis of renal glycosuria can be established definitely.

The essential cause of renal glycosuria is unknown. Autonomic instability may be a factor in its etiology as it has been shown that individuals with autonomic imbalance are hypersensitive to phlorhizin. Whatever the cause may be, inefficiency of the mechanism for tubular reabsorption of glucose is the fundamental defect that determines the occurrence of glycosuria in this condition. From this standpoint it resembles phlorhizin glycosuria. No metabolic disturbance occurs in subjects with renal glycosuria as long as the carbohydrate intake is adequate to compensate for the amount lost in the urine. Deprivation of carbohydrate may cause hypoglycemia, increased sensitivity to insulin and ketosis more readily than in normal subjects.

Glycosuria of Pregnancy. Glycosuria, occurring during a normal, uncomplicated pregnancy, appears to be due to lowering of the "renal threshold" since it is associated with no elevation of blood sugar. It is observed in as many as 10-15 per cent of all normal pregnant women, particularly in the later months and more frequently in primigravidae than in multigravidae. Pregnancy glycosuria is ascribed by some observers to a decreased carbohydrate tolerance resulting from the hypertrophy of the pituitary gland which occurs during that period. Lactose, contrary to popular opinion, is never normally present in the urine during pregnancy, under normal conditions of lactation, physiologic lactosuria occurring only during the period of lactation.

"Alimentary" Glycosuria. Opinion is divided regarding the metabolic status of so-called "alimentary" glycosuria. The term is employed to designate the urinary excretion of glucose by certain apparently normal individuals after the ingestion of excessive amounts of cane sugar, glucose or, at times, starch. It is evident that the occurrence of glycosuria under such circumstances must be due either to a "lowering of the renal threshold" for glucose or to the absorption of glucose from the intestine at a rate too rapid to allow of its adequate removal from the circulation by the liver (e g., in hyperthyroidism).

Woodyatt and his associates have shown that the normal individual can utilize glucose injected intravenously in amounts up to 0.8 Gm. per kilogram of body weight per hour; when this rate is exceeded glycosuria occurs. It has also been demonstrated that the absorption of glucose from the intestine proceeds normally at a maximum rate of 1.8 Gm. per kilogram of body weight per

hour, regardless, within wide limits, of the quantity of sugar ingested. Consequently, if the liver removes a minimum of 1.0 Gm. per kilogram per hour, allowing 0.8 Gm. to pass into the general circulation, glycosuria should not be expected to occur in normal individuals. In the absence of any abnormality of hepatic or tissue glycogenic function, alimentary glycosuria might be explained upon the basis of increased permeability of the intestinal mucosa for glucose resulting in its absorption at a rate more rapid than can be adequately handled by the liver; it therefore reaches the tissues, including the kidneys, in excessive amount, with the result that a portion is eliminated in the urine. This hypothesis does not necessarily imply the existence of venous hyperglycemia, for, tissue utilization being unimpaired, slight grades of arterial hyperglycemia may possibly be corrected and the concentration of glucose in the blood leaving the tissues may be within normal limits. This possibility has been supported by Friedenson in studies of capillary and venous blood sugar tolerance curves in benign glycosuria. Some authorities believe that alimentary glycosuria, in most instances, is in reality renal glycosuria. Others maintain that many such cases are dependent upon some disturbance of intermediary carbohydrate metabolism originating in the liver, endocrine glands or tissues (muscles). No such disturbance can be demonstrated satisfactorily in most cases.

Glycosuria in Glomerulonephritis and Nephrosis. Glycosuria occurs at times in a considerable proportion of patients with glomerulonephritis, nephrosclerosis and nephrosis.¹⁶ In a great majority of such individuals glucose is excreted in the urine in larger quantities than in normal subjects, gross glycosuria occurring in from 30-50 per cent of all cases, the frequency and degree increasing with increasing severity of the renal lesion. In some patients with glomerulonephritis and nephrosclerosis glycosuria is associated with fasting hyperglycemia or diminished glucose tolerance or both (see pp. 31, 43). However, in many such cases and in individuals with nephrosis the glycosuria appears to be dependent upon a decrease in the renal threshold for sugar. This increased elimination of glucose in the urine, particularly in patients with nephrosis, may possibly be due to failure of the renal tubules to reabsorb glucose from the glomerular filtrate as a result of the degenerative changes in the renal tubular epithelium. In such cases the urine may contain more than 1 per cent of glucose after the ingestion of a carbohydrate rich meal (p. 405).

Hyperglycemic Glycosuria. The occurrence of glycosuria in association with hyperglycemia is readily understood. If one

accepts the normal "renal threshold" value as being 140-160 mg. of glucose per 100 cc. of blood, the elimination of glucose in the urine may be expected in the presence of higher blood sugar levels. The fact must be kept in mind, however, that the renal threshold may exhibit rather wide variations in different individuals and under different conditions. Obviously, the causes of hyperglycemia are potential causes of glycosuria. These include the following:

Diabetes Mellitus (p. 332). Diabetes mellitus is the most frequently observed individual cause of glycosuria dependent upon hyperglycemia. The view that glycosuria always indicates diabetes mellitus is, however, erroneous, even in the presence of hyperglycemia. John, in an illuminating analysis of 398 patients with glycosuria, found that only approximately 37 per cent were true diabetics. Diabetes was the cause of glycosuria in only 29.9 per cent of individuals under twenty years of age presenting this symptom. As has been mentioned previously, glycosuria is not uncommon in the later months of pregnancy. Its occurrence in the early months should lead to a rigid investigation of the possibility of the existence of diabetes mellitus. The differential diagnosis between diabetes and other conditions causing hyperglycemic glycosuria entails an investigation of blood sugar tolerance curves, the arterial-venous blood sugar difference, the serum phosphate curve and respiratory quotient variations following glucose ingestion, the plasma cholesterol concentration and the basal metabolic rate (hyperthyroidism). Further details will be considered in the section on diabetes mellitus (p. 332).

Hyperthyroidism. Glycosuria occurs in 25-35 per cent of patients with hyperthyroidism. Its incidence is somewhat higher in cases of primary (exophthalmic goiter) than of secondary hyperthyroidism (toxic adenoma). Associated with manifestations of hyperthyroidism, particularly an increase in the basal metabolic rate, it is usually readily distinguishable from other causes of glycosuria and hyperglycemia. It must be recognized that diabetes and hyperthyroidism may co-exist and that hyperthyroidism is likely to act as a predisposing cause of diabetes. Glycosuria in this condition may be due in part to an increased rate of absorption of glucose from the intestine (alimentary glycosuria) as well as to lowered tolerance.

Hyperpituitarism. Glycosuria occurs in 25-40 per cent of patients with acromegaly at some time during the course of the disease. It occurs also as a part of the characteristic clinical picture of pituitary basophilism (Cushing's syndrome) but is not present in all cases

Adrenal Hyperfunction. Glycosuria may occur during periods of excessive nervous strain, severe exercise and emotional excitement (fear, anger, anxiety). It is also a feature of the clinical picture of Cushing's syndrome due to adrenal cortical hyperplasia or tumor.

Miscellaneous. Glycosuria may occur in hemochromatosis, advanced pancreatitis and severe hepatocellular damage, in the latter usually with a normal fasting blood sugar concentration and only after a high carbohydrate intake. It is seen at times with head injuries or other causes of increased intracranial pressure (brain tumor, abscess, hemorrhage), hypothalamic lesions, ether or chloroform anesthesia, narcosis by morphine or barbiturates, asphyxia, acidosis, acute and chronic infections, coronary artery occlusion, advanced malignancy, simple obesity and essential hypertension. It has also been observed after administration of caffeine, diuretin, strychnine, bichloride of mercury and chromates. In these conditions, the persistence and severity of glycosuria are related usually to the persistence and severity of the accompanying hyperglycemia (pp. 27-32), except in the presence of renal tubular damage. Consequently, it is usually transitory and of mild degree (less than 1 per cent) in conditions other than diabetes mellitus, hemochromatosis and Cushing's syndrome.

LEVULOSURIA^{11,17}

Levulose (fructose) reduces metallic oxides in alkaline solution, is fermentable by bakers' yeast and yields an osazone with phenylhydrazine which is morphologically identical with glucosazone. Levulose may be identified in the urine by the following methods:

(1) Gas production with *Monilia krusei* but not with *Monilia balcanica*, to exclude glucose.

(2) Characteristic osazone crystals and positive Seliwanoff (resorcinol-HCl) or Borchardt reaction, to exclude glucose. The presence of nitrites or indican in excess interferes with the development of the characteristic yellow color of Borchardt's reaction. Glucose, in large amount (2 per cent), may yield a positive Seliwanoff reaction.

(3) Rotation of polarized light to the left in the absence of other levorotatory substances as conjugate glucuronates and beta-hydroxybutyric acid.

Levulose may appear in the urine under the following circumstances:

(a) In severe cases of diabetes mellitus, always in association with glucose.

(b) Alimentary levulosuria, following the ingestion of large quantities of levulose, particularly in patients with hepatic insufficiency. This has been utilized as a test of hepatic function but is unsatisfactory; approximately 10 per cent of normal individuals eliminate levulose in the urine following the ingestion of 100 Gm. of levulose.

(c) Essential levulosuria, implying the occurrence of levulosuria in the absence of the above-mentioned factors, is a rare condition. Silver regards it as a specific, probably inborn error of metabolism, localized primarily in the liver where, he believes, a specific enzyme deficiency exists, resulting in impaired ability to fix fructose as glycogen. The metabolism of other carbohydrates is undisturbed. A few cases have been reported in which there was a total absence of tolerance for levulose, the sugar being eliminated if any whatsoever was ingested. Heeres and Vos state that, regardless of the amount ingested, about 14 per cent is eliminated. Insulin has no influence upon this condition; no rise in the respiratory quotient follows the administration of levulose to such individuals, indicating failure of utilization of that sugar. It is said that rectal administration produces more severe levulosuria than when given by mouth.

PENTOSURIA

Pentoses reduce metallic oxides in alkaline solution and are nonfermentable by bakers' yeast. They may be identified in the urine by the following methods:

(1) Positive Bial Reaction (Orcinol-HCl).

(2) Positive Benedict reaction and negative fermentation test with bakers' yeast in the absence of lactose and nonsugar, reducing substances (conjugate glycuronates).

(3) Characteristic pentosazone crystals with phenylhydrazine.

(4) Positive Benedict reaction, gas production with *Bacillus coli communis* and *B. paratyphosus* B (to exclude lactose) and no fermentation by *Monilia tropicalis* (to exclude galactose).

Pentose may appear in the urine under the following circumstances:

(a) Alimentary pentosuria.

This is a temporary condition, occurring in normal individuals after the ingestion of large quantities of fruits which have a high pentose content (prunes, cherries, grapes, plums).

It is of no clinical significance apart from the fact that it may be mistaken for glycosuria because of a positive copper-reduction test.

(b) Diabetes mellitus (Some cases).

(c) Essential pentosuria (Chronic pentosuria).

This is a relatively rare and extremely interesting condition which is analogous to essential levulosuria. Pentoses are more or less constantly present in the urine, the quantity excreted bearing no relation to the amount ingested. It is of no known clinical significance, since the utilization of other carbohydrates is unimpaired. It appears to be familial and hereditary in nature. As in the case of alimentary pentosuria, its chief importance lies in the possibility of mistaking it for glycosuria.

LACTOSURIA

Lactose reduces metallic oxides in alkaline solution (Benedict, Fehling, etc.) and, in about 15 per cent of cases, is fermented by bakers' yeast. It may be identified in the urine by the following methods:

(1) Positive Benedict test, gas production with *B. coli* communis and no gas production with *B. paratyphosus* B (to exclude pentose).

(2) Positive mucic acid test and negative phloroglucinol-HCl reaction (Tollens) to exclude galactose.

(3) Characteristic lactosazone crystals with phenylhydrazine. This test is usually unsatisfactory.

(4) If positive Benedict test and no fermentation with bakers' yeast, negative Bial test to exclude pentose.

Lactosuria occurs in a considerable proportion of women during the period of lactation. It does not occur normally during pregnancy, under normal conditions of lactation. The lactosuria of lactation must be regarded as physiologic and has no apparent clinical significance.

GALACTOSURIA

Galactose reduces metallic oxides in alkaline solution and is fermented by most samples of bakers' yeast although usually not as actively as are glucose and fructose. It may be identified in the urine by the following methods:

(1) Positive mucic acid test to exclude all other reducing substances except lactose, and positive phloroglucinol-HCl reaction (Tollens) to exclude lactose.

(2) Positive Tollens reaction and no absorption bands upon spectroscopic examination (to exclude pentose and glycuronic acid).

MALTOSURIA

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Chapter II

Protein Metabolism

DIGESTION AND ABSORPTION^{7,123}

INGESTED proteins undergo digestion in the stomach and small intestine. This process consists essentially in hydrolysis into their constituent amino acids, with proteoses, peptones and polypeptides as intermediary products.

The factors in gastric juice important in this connection are (1) hydrochloric acid, (2) pepsin, a proteolytic enzyme, and (3) rennin, a milk-curdling proteolytic enzyme. The functions performed by HCl are: (a) conversion of protein to acid metaprotein; (b) activation of pepsinogen to pepsin; (c) provision of an optimum pH for peptic activity (about 1.5 to 2.2, varying somewhat for different proteins). Under optimum conditions *in vitro*, pepsin is capable of splitting proteins to the stage of amino acids, but under normal conditions of gastric emptying (p. 488), there is very little digestion beyond the stage of peptones, 45-70 per cent of the ingested protein being in the form of proteose at the time it leaves the stomach. Rennin splits the casein of milk into a proteose- or peptone-like body and soluble paracasein. The latter is converted to insoluble calcium paracasein, which undergoes peptic digestion. Gastric rennin is of particular importance in digestion in infants, in whom it is present in relatively large amounts and pepsin in only rather small amounts.

Digestion of protein and protein products in the intestine is continued by proteolytic enzymes of the pancreatic and intestinal secretions. Pancreatic juice contains trypsinogen, chymotrypsinogen and carboxypolypeptidase, the first two being activated by enterokinase, an enzyme secreted by the intestine. Carboxypolypeptidase breaks down polypeptides to simpler peptides and amino acids, whereas the other two enzymes act on native proteins to form proteoses, peptones, polypeptides, simpler peptides and amino acids, depending upon the length of time the reaction is allowed to proceed. In addition to enterokinase, the intestinal juice contains dipeptidase and aminopolypeptidase (formerly jointly termed erepsin), which split polypeptides to amino acids.

Nucleoproteins, constituents of all nuclear material, are split in the stomach by peptic digestion into protein and nuclein, the latter being hydrolyzed in the intestine by trypsin to protein and nucleic acid. The protein thus formed undergoes digestion in the stomach and intestine as outlined above. The nucleic acid is converted, by the action of nucleases in the lumen and wall of the intestine, to nucleotides (purine and pyrimidine), which are hydrolyzed by phosphatase in the intestine into phosphoric acid and the nucleosides, adenosine and guanosine, and pyrimidine substances. These are absorbed from the upper intestine, the portion escaping absorption undergoing bacterial decomposition with the liberation of ammonia.

Under ordinary conditions, protein is absorbed from the alimentary tract practically only in the form of amino acids, absorption occurring in the small intestine, chiefly into the portal circulation but possibly also, to a small extent, by way of the lacteals into the thoracic duct. Comparatively minute amounts of polypeptides and also of certain native proteins, such as raw egg albumin, may be absorbed as such under certain circumstances. This may be of importance in connection with food idiosyncracies (allergy)⁹³ These substances cannot be utilized for synthesis of body proteins without first being broken down into amino acids and, if they escape such conversion, they may be excreted unchanged in the urine.

INTERMEDIARY METABOLISM^{7,123}

Amino acids entering the circulation normally undergo one or more of the following changes:

Storage in Tissues. Amino acids are rapidly removed from the circulating blood by the tissues, particularly the liver and muscles, the latter being less active than the former in this regard.¹¹⁸ This tissue storage is only temporary, the amino acids being either synthesized into new substances or broken down further.

Slight Excretion in Urine.

Synthesis of New Nitrogenous Substances. New tissue proteins must be formed in order to provide for normal growth in the immature organism and for replacement of tissue protein destroyed in the course of normal catabolic processes in the adult. These have their origin in the circulating amino acids. The same is true of the globin fraction of hemoglobin and of the plasma proteins (albumin, globulin, fibrinogen) (p. 75). Amino acids also enter into the formation of enzymes, creatine, glutathione and many other essential substances, including certain

hormones of protein nature, such as insulin, thyroxin and the anterior pituitary hormones. The participation of specific amino acids in the synthesis of certain vital factors is illustrated by the relation of tyrosine to thyroxin and epinephrine and of glycine and cystine to the bile acids, glycocholic and taurocholic acids.

Catabolism. The amino acids that do not undergo the changes mentioned above are broken down into simpler substances. The first phenomenon in this degradation process is deamination, the split-off amino groups forming ammonia, which is eventually converted to and excreted as urea (p. 78). There is strong evidence that deamination takes place to a large extent in the liver,¹² but there is also evidence that it may occur in the kidneys and intestinal wall.^{67,73} Ammonia formed in the kidneys (tubular epithelium) may be utilized for the conservation of fixed base (p. 275).

The non-nitrogenous (fatty acid) residue after deamination may be metabolized as follows: (a) it may be oxidized to CO_2 and H_2O to supply energy; (b) the residue of certain of the amino acids (alanine, glycine, serine, cystine, aspartic, glutamic and hydroxyglutamic acids) may be converted to glucose and subsequently be burned or transformed to glycogen or fat; (c) ketogenic amino acid residues (leucine, tyrosine, phenylalanine) may form ketone bodies; (d) reamination may occur, with consequent retransformation into amino acids. It has been shown that glycine, alanine, arginine and glutamic acid can be synthesized in this manner. Glucose formed from protein constitutes 58 per cent, by weight, of the amount ingested, and fatty acids 46 per cent. The influence of the anterior pituitary and adrenal cortex upon gluconeogenesis and ketone body production is discussed elsewhere (pp. 17-23).

The catabolism of the sulfur-containing amino acids, cystine and methionine, involves other features which are considered elsewhere (pp. 125, 212). Suffice it to state here that the sulfur of these compounds (organic S) is largely oxidized to inorganic sulfate in the liver and is excreted in this form in the urine. The small portion that escapes oxidation may be excreted as neutral sulfur.

Purine nucleotides and nucleosides, after being absorbed from the intestine, undergo hydrolysis and oxidation, probably largely in the liver, but also perhaps in other tissues. Hydrolysis results in the formation of the purine bases, adenine and guanine, which are oxidized to hypoxanthine, xanthine and, ultimately, uric acid, which is the chief end-product of nucleoprotein catabolism in man. The pyrimidine bases, thymine, cytosine and uracil, are broken down into urica. There is some question as to whether

uric acid is destroyed in the human body, but the weight of available evidence does not support this possibility.⁵

EXOGENOUS AND ENDOGENOUS PROTEIN METABOLISM

The term "exogenous" is applied to the metabolism of ingested protein, whereas the metabolism of tissue proteins is termed "endogenous." Urea is a product chiefly of exogenous, but also of endogenous metabolism; uric acid is equally representative of both forms; creatinine, on the contrary, is derived from creatine. The intermediate stages of endogenous protein metabolism, such as deamination, urea formation, etc., are identical with those occurring in the metabolism of ingested proteins.

Because of the constancy of excretion of creatinine under normal conditions, the urine creatinine came to be regarded, according to the classical concept of protein metabolism, as an index of the rate of endogenous protein metabolism. It is now known that the amount of urinary creatinine is a measure not of the activity of tissue protein catabolism, but of the level at which the body maintains its phosphocreatine.¹² Recent studies⁹⁸ have shown also that nearly all proteins of the body, including plasma proteins, continually undergo breakdown and synthesis, more than half the protein of the liver and intestinal mucosa being broken down and resynthesized in ten days; the turnover is slower in the muscles and erythrocytes. Active resynthesis occurs even during periods of starvation, and breakdown of protein in one organ may be accompanied by synthesis in others.

NITROGENOUS CONSTITUENTS OF THE BLOOD

As a result of the processes mentioned above, the blood plasma normally contains a number of nitrogenous substances. These may be classified as (1) proteins and (2) nonprotein nitrogenous substances.

Plasma Proteins.^{19,77,112,119,123} The proteins commonly included under this designation are albumin, globulin and fibrinogen. Blood serum contains only the first two, fibrinogen being converted to fibrin and incorporated in the clot during the process of coagulation. There is evidence that there are two albumins and three globulins in normal plasma or serum. On the basis of chemical methods the serum globulin may be separated into pseudoglobulin and euglobulin; by electrophoresis, three fractions have been differentiated, alpha, beta and gamma globulins.

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slightly higher for heparinized and slightly lower for oxalated plasma (despite its fibrinogen content) than for serum.

The chief functional significance of the plasma proteins lies in the fact that because of the relative impermeability of the capillary walls to colloids, the colloid osmotic pressure of the plasma proteins, which normally ranges from 24-30 mm. Hg, plays an important part in the maintenance of the normal distribution of water between the blood and the tissues. The osmotic pressure of blood plasma is approximately 7 atmospheres, being dependent upon its crystalloid and colloid content. Because of the fairly high degree of diffusibility of the crystalloid constituents, the concentrations of these substances in plasma and tissue fluids are practically identical; minor differences exist because of the effect of a Donnan equilibrium. It may therefore be considered that, in the resting state, the crystalloid osmotic pressure of the plasma is balanced by that of the tissue fluids.

There is considerable disparity, however, between the protein content of plasma and that of tissue fluid, the latter varying in different situations due to varying degrees of capillary permeability, as demonstrated by Starling. Thus the lymph flowing from the liver contains 6-7 Gm. per cent of protein, that from the extremities only 2-3 Gm. per cent. Because of this difference, the colloid osmotic pressure of the plasma (24-30 mm. Hg) exerts a force which tends to direct the flow of water from the tissues into the blood. This force, the colloid osmotic pressure or oncotic pressure of the plasma, is a function largely of the molecular size of the various proteins. It is influenced consequently much more by albumin than by globulin, 2.4-3.9 times as much, according to different observers,^{44, 54} because of the smaller size of the albumin molecule (molecular weight of serum albumin about 70,000; serum globulins, 150,000-190,000). Thus, albumin, comprising about 60 per cent of the total proteins, is responsible for about 80 per cent of the oncotic pressure of human plasma.¹⁹ By direct measurement, the colloid osmotic pressure of normal plasma or serum has been found to vary from 280 to 470 mm. H₂O. Under normal conditions, this force, which tends to direct the flow of water from the tissues into the blood, is counterbalanced by the opposing force of the capillary blood pressure (13-35 mm. Hg). Because of the drop in blood pressure from the arteriolar to the venous end of the capillary, fluid tends to pass from the blood to the tissues on the arteriolar side of the capillaries and from the tissue spaces to the blood at the venous end. Passage of fluid in the latter direction is also favored by the increased colloid osmotic pressure of the blood plasma at the

The plasma proteins are synthesized from amino acids; just as are other tissue proteins,^{79,76} the exact site of their formation being open to question.^{77,123} Fibrinogen is probably formed entirely in the liver. It seems likely that the albumin and globulin are formed in the liver, the blood-forming organs, the spleen and perhaps the intestinal mucosa. Globulins may be formed by plasma cells and by cells of the reticulo-endothelial system generally. If it is assumed that these proteins are formed outside the circulation, it is obvious that they must enter the blood stream in situations where the capillaries are sufficiently permeable to permit their ready diffusibility. The important role of the liver in the synthesis of serum albumin is illustrated by the markedly diminished capacity for its formation exhibited by Eck-fistula dogs and by animals with liver damage induced by hepatotoxic agents.⁶⁶ It has been found that normal dogs, under optimum conditions, are capable of regenerating about 90 per cent of their total plasma protein weekly.

There is a reserve store of plasma protein-building material, probably largely in the liver, which may be drawn upon for emergency regeneration of these substances. Whipple and his associates⁷⁷ believe that a state of dynamic equilibrium exists between these reserve stores and the plasma proteins (albumin and globulin), hemoglobin and cellular proteins. If the requirement for protein to build new cell protein, hemoglobin or plasma protein exceeds the exogenous supply, the reserve store will be drawn upon. It also appears that the plasma proteins can be utilized for maintenance of general nutrition and nitrogen equilibrium.

Fibrinogen. The fibrinogen content of normal plasma has been variously estimated as from 200-600 mg. per 100 cc. It is probable that the true normal range is within much narrower limits, the values most commonly obtained being 200-400 mg. per 100 cc. Its most important function is in connection with the phenomenon of blood coagulation, its presence in adequate amount being essential for the normal clotting of blood. In this process it is transformed into fibrin by the action of thrombin.

Albumin and Globulin. The total serum protein concentration is 6-8 Gm. per 100 cc., of which 3.6-5.6 Gm. is represented by albumin and 1.3-3.2 Gm. by globulin (average pseudoglobulin 1.7 Gm.; euglobulin 0.7 Gm.). At birth, the average total serum protein concentration has been found to be 5.7 Gm., falling to 5.33 Gm. during the first month and gradually rising to reach the adult level of 6.94 Gm. at the fourth month.¹¹³ The normal albumin:globulin ratio is 1.5-2.5:1. The normal values for serum are practically identical with those for plasma, being

has been shown that another mechanism may operate under physiologic conditions:⁶³ (1) ornithine + carbon dioxide + ammonia form citrulline and water; (2) citrulline + ammonia form arginine; (3) hydrolysis of arginine by arginase forms urea and ornithine, the latter becoming available for another cycle.

The blood urea nitrogen may rise markedly following a meal high in protein and tends to maintain a relatively higher level in individuals on a high protein diet than in those on a low protein intake. It is essential, therefore, in making comparisons between blood urea values, that a standard diet be administered and that the specimen be taken in the fasting state.

Urea is an extremely diffusible substance and, as such, exists in all body fluids in practically the same concentration. Thus it is present in the spinal fluid, saliva, exudates and transudates in approximately the same amount as in blood. It is eliminated chiefly in the urine, but considerable amounts may be lost through the skin if perspiration is active.

Uric Acid. The uric acid content of normal blood is 2-4 mg. per 100 cc. and of plasma or serum, collected anaerobically, 2-6 mg. per 100 cc. The latter values are more significant clinically than the former.⁶⁴ Uric acid is, in man, the chief end-product of exogenous and endogenous nucleoprotein metabolism. Folin has reopened the formerly much disputed question as to whether or not uric acid can be further utilized or destroyed in man as in most other mammals. It appears probable that certain organs, particularly the kidneys, may be capable of temporarily storing relatively large amounts of uric acid which may subsequently be liberated and destroyed in the tissues, the liver being of great importance in this connection. If such destruction does occur, the final end-product has not been determined. The consensus at present is that destruction of uric acid probably does not occur in humans.

The concentration of uric acid in the blood is normally affected but slightly, if at all, by the ingestion of purine-rich foods, and bears no direct relation to the level of total nonprotein nitrogen or urea. It is normally excreted practically entirely by the kidneys.

Creatinine. The creatinine content of normal blood ranges from 1 to 2 mg. per 100 cc. It is the anhydride of creatine, which is present in the muscles. Creatinine is readily diffusible and is excreted largely by the kidneys.

The methods employed for the determination of blood creatinine are nonspecific in nature. However, whatever may be the exact nature of the substances in the blood which respond to the reaction for creatinine, so remarkable is their constancy in

venous end of the capillary, which results from the increased concentration of plasma proteins incident to the loss of fluid in the proximal end of the capillaries. Under conditions of active tissue metabolism the osmotic pressure of the tissue fluids is raised by the decomposition of carbohydrates, proteins and fats into relatively much smaller molecules, thus temporarily aiding in the abstraction of water from the blood plasma.

Antibodies, developed in response to infection, are in large part constituents of or associated with the globulin fraction, chiefly gamma globulins but perhaps also beta globulins. The so-called "mid-piccc" of complement appears to be a beta globulin and the "end-piccc" and "fourth component" of guinea pig complement appear to be alpha globulins.

The plasma proteins play a minor role in the maintenance of the normal acid-base equilibrium of the blood (see p. 270).

A slight and transitory increase in the plasma protein concentration may result from physiologic factors, such as prolonged hyperventilation, vigorous and protracted muscular exercise, blood stasis due to chilling, marked sweating and inadequate fluid intake.

Nonprotein Nitrogen. The nonprotein nitrogen (NPN) of the blood, that portion of the nitrogenous substances not precipitated by the usual protein precipitants, includes urea, uric acid, amino acids, creatine, creatinine, ammonia, and a fraction designated "undetermined nitrogen," which consists perhaps of polypeptides and other aggregations of amino acids.¹⁰ From a metabolic standpoint, the nonprotein nitrogenous constituents of the blood are usually of greater clinical interest than the plasma proteins since they represent products of the intermediary metabolism of ingested and tissue protein. The normal NPN of whole blood is usually 25-35 mg. per 100 cc., figures as high as 40 mg. occurring occasionally, and as low as 16 mg. at times in normal pregnancy.

Urea. The blood urea normally ranges from 20 to 35 mg. per 100 cc., the urea *N* being 9-17 mg. (46.6 per cent of the total urea molecule). The extreme normal limits under conditions of very low to very high protein intake are 5-23 mg. urea *N* per 100 cc. Values of 5-12 mg. are common in the last months of normal pregnancy. Urea is the chief end-product of protein metabolism and is probably formed normally largely if not entirely in the liver. It has been assumed for a long time that its formation involves successive processes of (1) deamination of amino acids, (2) formation of ammonium carbonate (ammonia + carbon dioxide + water) and (3) dehydration to ammonium carbamate and finally to urea. More recently it

Urea. The quantity of urea excreted in the urine, being to a considerable degree the result of exogenous protein metabolism, depends largely upon the protein intake. The percentage of the total urinary nitrogen represented by urea likewise varies directly with the amount of protein ingested. The lower the protein intake the greater is the relative proportion of products of endogenous metabolism such as creatinine and uric acid, although the actual amount of the former is unaltered and the latter greatly decreased. The relative proportion of ammonia nitrogen is likewise greatly increased under such circumstances. Upon a high protein intake (120 Gm.), urea may constitute approximately 90 per cent of the total urine nitrogen; upon an intake of 6 Gm. of protein this figure is reduced to about 60 per cent (Folin). With an average diet, approximately 30 Gm. of urea are eliminated in twenty-four hours, constituting about 50 per cent of the total urinary solids (see Table 3, p. 127).

It is evident that the total urinary nitrogen, as well as the urea nitrogen, depends upon the nitrogen intake and, to be of any clinical significance, must be regarded in its relation to the latter. Furthermore, since nitrogen balance may be established with varying levels of nonprotein nitrogen in the blood, the isolated determination of total urinary nitrogen or urea nitrogen is of little clinical value under ordinary circumstances.

Addis and Drury have demonstrated that the ratio,

$$\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc. blood}} = 50,$$

is quite constant within rather narrow limits regardless of moderate variations in urine volume. This ratio, which may be readily determined, may be of clinical significance in the estimation of renal function. Möller, McIntosh, Van Slyke and their associates, in studying the urea-eliminating function of the kidney, have utilized the principle of "blood urea clearance," an expression of the volume of blood which is cleared of urea per minute by renal elimination. It was found that, in the presence of a urine volume output of less than 2 cc. per minute, the blood urea clearance may be calculated according to the formula,

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where U signifies the urea concentration in the urine, B the urea concentration in the blood and V the urine volume in cc. per minute. This is termed the "standard blood urea clearance" and is normally 54 cc. If the urine volume output is greater than

health and so specific are variations in their concentration in disease that the question as to whether or not this nitrogen fraction is in reality creatinine is of more academic than practical clinical interest.

Amino Acids. The amino acid nitrogen content of normal blood is 5-8 mg. per 100 cc., the average concentration in plasma being about 3.5 mg. per 100 cc. It is derived from that portion of the amino acids absorbed from the intestine which has escaped deamination and synthesis into protein in the liver, and from amino acids resulting from breakdown of tissue protein which have not as yet undergone deamination or synthesis. They are eliminated as such in the urine. A slight rise in the amino acid concentration of the blood occurs following the ingestion of proteins, and insulin causes a fall.

Ammonia. The ammonia nitrogen content of normal blood is 0.1 to 0.2 mg. per 100 cc. and is of little clinical significance. It is produced as a result of the deamination of amino acids by the liver and represents largely that portion which has not been converted into urea.

Undetermined Nitrogen (Rest Nitrogen). After all of the known nonprotein nitrogenous constituents of the blood have been determined, including those mentioned above and others, such as creatine (2-7 mg. per 100 cc.) of no apparent clinical significance, there remains a considerable amount of nonprotein nitrogen of undetermined composition termed "rest nitrogen" or "undetermined nitrogen." This may constitute as much as 45 per cent of the total nonprotein nitrogen, ranging normally from 5-18 mg. per 100 cc. and residing chiefly in the corpuscles. Since its nature is unknown, its metabolic significance and its derivation are conjecturable. However, it may bear an important relationship to the toxic manifestations of certain disease states such as eclampsia and uremia.

NITROGEN ELIMINATION

Under perfectly normal conditions the urine contains an extremely small amount of albumin which usually cannot be detected by ordinary qualitative methods. The excretion of albumin by essentially normal individuals (functional, orthostatic or adolescent albuminuria) will be considered in the discussion of albuminuria. From a practical viewpoint, with certain reservations, therefore, it may be stated that protein is not normally present in the urine.

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etc.), increase the uric acid content of the urine. According to Mendel, 10-15 per cent of the caffeine is converted into uric acid. An increase may also follow administration of salicylates, cinchophen and neocinchophen.

Creatine and Creatinine. Creatine, probably a derivative of glycine, guanidine or guanidine-acetic acid, is not a waste product, but is of fundamental importance in the phenomenon of muscle contraction (as phosphocreatine). The capacity of the muscles for contraction is related to their phosphocreatine content, the force of contraction is proportional to the extent of breakdown of the latter, and restoration of excitability in the muscle after contraction is dependent upon resynthesis of the hydrolyzed phosphocreatine. Creatine is present in the urine of prepuberal children (4.2 mg. per kilogram daily), during pregnancy and the puerperium (two to three weeks) and occasionally in normal, nonpregnant women, but is absent from the urine of normal males on a balanced diet. It may appear in the urine of normal subjects maintained on a very low carbohydrate intake and during fasting periods (up to 100 mg. creatine daily), and is believed to be derived from excessive catabolism of muscle tissue.

When 1.32 or 2.64 Gm. of creatine hydrate (equivalent to 1 and 2 Gm. of creatine) are ingested by normal adults, about 80 per cent is retained by males and about 70 per cent by females, about 20 per cent and 30 per cent respectively being excreted in the urine during the next twenty-four hours. This forms the basis of the creatine tolerance test, which is of value clinically in demonstrating excessive excretion of creatine in the absence of spontaneous creatinuria under ordinary dietary conditions. It is also of value in affording a quantitative index of the functional state of the muscles in various myopathies and other conditions (p. 124).

Creatinine (creatine anhydride) is a waste product derived from creatine, and is therefore usually of endogenous origin. Normal adult males excrete 1.5-2 Gm. and females 0.8-1.5 Gm. daily, the amount eliminated being uninfluenced by the urine volume or the character of the diet if the latter is adequate and contains no creatinine or creatine. Folin found that upon a diet containing 118 Gm. of protein and 2786 calories, 0.58 Gm. of creatinine was eliminated, constituting 3.6 per cent of the total urinary nitrogen; upon a diet containing 6 Gm. of protein and 2153 calories, 0.60 Gm. of creatinine was eliminated, constituting 17.2 per cent of the total urinary nitrogen (See Table 3, p. 127). Because of this constancy of elimination, the determination of the total creatinine excretion has been employed as a means of checking the accuracy of twenty-four-hour urine col-

2 cc. per minute, the term "maximum blood urea clearance" is employed, the formula being

$$\frac{UV}{B}$$

the normal value for which is 75 cc. The important subjects of the mechanism of renal excretion of urea and the "clearance" concept are considered in detail elsewhere (Renal Function, pp. 349, 372).

Although the quantity of urea eliminated varies considerably according to the protein intake, the percentage of urea in the urine is quite constant under normal conditions. MacLean observed that the urine excreted during the second hour following the ingestion of 15 Gm. of urea in 100-150 cc. of water normally contains 2 per cent or more of urea. This observation has been utilized, variously modified by different authors, as the basis for several urea concentration tests of renal function.

Uric Acid. The quantity of uric acid present in the urine of normal individuals depends upon the amount of nucleoprotein ingested (exogenous uric acid) and upon the amount formed as a result of the metabolism of tissue nucleoprotein (endogenous uric acid). On a purine-free diet the average daily excretion is 0.1-0.5 Gm. (Hawk). This is derived entirely from endogenous nucleoprotein metabolism and may be increased by exercise. If a normal individual is kept upon a purine-free diet the quantity of uric acid in the urine may be varied by altering the protein content and caloric value of the diet. Thus, a high protein or high caloric intake, by stimulating endogenous metabolism, increases the urinary uric acid; conversely, a low protein or low caloric intake decreases the uric acid content of the urine (see Table 3, p. 127). On a high purine diet (meat, liver, kidney, sweetbreads, leguminous vegetables, etc.) the uric acid output may be as much as 2 Gm. per day; for adults on a mixed diet the average daily excretion is about 0.7 Gm. (Hawk). During the first few days of life, the urine contains relatively large quantities of uric acid, constituting 7-8 per cent of the total nitrogen, as compared with 1-2 per cent in adults.⁹³ This is important in relation to the development of urate infarcts in infants.

As much as 50 per cent of ingested nucleoprotein may be recovered from the urine, the increased elimination occurring promptly and continuing for twenty-four to forty-eight hours after discontinuing the administration of purine foods. The remainder is either destroyed or utilized in the body or is eliminated in some form which is not at present recognized. Tea, coffee and cocoa, which contain methyl purine bodies (caffeine,

the same time, the maximum conservation of fixed base. Exceptions to this rule include acidosis due to excess carbonic and phosphoric acids and that accompanying renal disease, in which the ammonia-forming function of the renal epithelium is impaired (p. 280). Urinary ammonia is increased in normal subjects following ingestion of acid-forming foods, mineral acids or their ammonium, calcium or magnesium salts, by a low carbohydrate intake (ketosis) and in normal pregnancy. It is decreased following ingestion of alkalis or base-forming foods and during the "alkaline tide" accompanying gastric secretion of free HCl (p. 274).

NITROGEN BALANCE

The term "nitrogen balance" is applied to the difference between the daily nitrogen intake and the daily nitrogen loss. Since the quantity lost in milk, sweat, saliva, hair, skin scales, etc., is usually negligible, only the urine and feces need be considered in this connection under ordinary circumstances. The normal adult excretes about 1.3 Gm. of nitrogen daily in the feces, which represents in part unabsorbed nitrogen of the food and in part nitrogen excreted into the bowel. If sweating is profuse, relatively large amounts of urea may be lost through this channel, and in patients with excessive vomiting or diarrhea the vomitus and feces may contain large amounts of incompletely digested protein and also nonprotein nitrogen derived from the gastro-intestinal secretions. A normal adult on a normal diet is in nitrogen equilibrium, the balance becoming positive (nitrogen retention) during a long period of adjustment to an increased protein intake. A positive balance is present physiologically in growing children, during pregnancy and during recovery from wasting diseases. The nitrogen balance is negative in normal subjects on an inadequate protein and carbohydrate intake, during prolonged, excessive muscular activity, during lactation (unless the protein intake is increased) and in the puerperium.

The nitrogen balance is negative when the protein, carbohydrate or total caloric intake is less than is required under existing conditions. This may occur in conditions accompanied by vomiting, diarrhea, fever or wasting (typhoid, tuberculosis, malaria, pneumonia, septicemia or bacteremia, malignancy, leukemia, diabetes mellitus), dehydration, excessive diuresis or purgation, hyperthyroidism, pituitary basophilism and adrenal cortical hyperfunction. A positive nitrogen balance may occur in acromegaly and after administration of testosterone or anterior pituitary extracts containing growth hormone.

lections. If marked variations in urinary creatinine are observed that do not depend upon corresponding changes in weight or surface area, one may assume that there has been some error in the collection of the urine specimens. Because of its origin from creatine, the quantity of creatinine excreted in the urine is an index of the level at which the body maintains its phosphocreatine and not of the rate of endogenous protein metabolism. The amount of creatinine excreted in the urine in twenty-four hours corresponds to about 2 per cent of the creatine in the body.

The term "creatinine coefficient" is used to indicate the number of milligrams of creatinine (plus creatine) nitrogen excreted per kilogram of body weight in twenty-four hours. Normal values range from 7.5 to 10 (20-26 mg. creatinine) for men and 5-8 (14-22 mg. creatinine) for women.¹⁰⁴ The creatinine coefficient may be assumed to be an index of the amount of active muscle tissue in the body. It is believed by some that muscular exercise has no influence in this connection, but others believe that although the daily elimination is constant, the urinary creatinine is increased during the period of muscular activity, being derived from creatine liberated during this period.

Amino Acids. Amino acids are eliminated in both the combined and the free state. In normal adults the total urinary amino acid nitrogen varies from 0.5 to 1.0 Gm. daily, constituting from 2 to 6 per cent of the total urinary nitrogen. The free amino acid nitrogen ranges from 0.1 to 0.15 Gm. daily, or 0.5-1.0 per cent of the total urinary nitrogen. It is normally dependent, as is the blood amino acid content, upon the proportion of amino acids which escape deamination or resynthesis to protein in the tissues.

Ammonia. The daily output of ammonia in the urine varies from 0.5 to 1.0 Gm., comprising 2-5 per cent of the total urinary nitrogen. As stated elsewhere (p. 346), urinary ammonia appears to be formed in the kidneys, being derived from amino acids through the activity of the epithelial cells of the renal tubules.¹⁰ The generally accepted view is that its production is regulated by the requirement of the body for excretion of acids which could not otherwise be eliminated in the urine without withdrawing from the body an excessive quantity of fixed base (Na, K, Ca, Mg).⁹⁰ According to some,¹⁶ production of ammonia by the kidneys is stimulated by and serves to neutralize the fixed acid residue that remains in the renal tubules after reabsorption of the alkaline "threshold" moiety from the glomerular filtrate.

From a practical viewpoint, urinary ammonia usually varies in accordance with the body's needs for excretion of acid with, at

(5) Constitutional Fibrinogenopenia. In this condition there may be delayed coagulation of blood or profuse bleeding after slight trauma, due to an inadequate amount of fibrinogen.⁹⁹

(6) Subnormal values have been observed occasionally in pernicious anemia, pellagra, scurvy, myelogenous leukemia and other disorders of the bone marrow.¹²⁴

Plasma Albumin and Globulin. The concentration of plasma albumin varies much more than does that of globulin. Albumin is affected much more readily than globulin by the protein intake, whereas globulin is regenerated more quickly after hemorrhage. The generalization may be made that the albumin fraction is rarely increased above normal and the globulin rarely decreased below normal.

Hypoproteinemia. There are several groups of clinical conditions in which a decrease in plasma albumin (normal 3.6-5.6 Gm. per 100 cc.) is of great practical significance. One of the most important functions of the plasma proteins is in the maintenance of the colloid osmotic pressure of the blood plasma. Any marked decrease in albumin is usually followed or accompanied by an increase in globulin (normal 1.3-3.2 Gm. per 100 cc.), apparently an attempt at compensation for the primary deficiency. As a result of these changes the albumin: globulin ratio, normally 1.5-2.5:1, is decreased or, in some cases, may be actually reversed. In some instances there may be an associated compensatory increase in fibrinogen, which, however, is usually present in too low concentration to be of practical significance in this connection.

Edema is the most prominent clinical feature of reduced plasma protein concentration. It is a consequence of the diminution in plasma colloid osmotic pressure which results in a decreased ability of the plasma to hold water. The increase in globulin and fibrinogen is incapable of compensating effectively for the decrease in albumin because, the size of their molecules being greater, they are much less effective than albumin in raising the colloid osmotic pressure.

Wells has found that the relation between the total protein and albumin concentrations and the osmotic pressure is sufficiently regular and definite to allow the use of an empirical formula for calculation of the osmotic pressure. He suggests the following formula, based upon his experimental observations: $P = C(21.4 + 5.9A)$, where P is the osmotic pressure in millimeters of water, C is the total protein concentration and A is the albumin concentration in grams per 100 cc. The standard error of the calculation is ± 5 per cent. This formula implies that the partial osmotic pressure of globulin depends upon the con-

ABNORMAL PROTEIN METABOLISM

Investigation of protein metabolism clinically is necessarily restricted almost entirely to the study of the protein and non-protein nitrogenous constituents of the blood and urine.

PLASMA PROTEINS

Fibrinogen. The plasma fibrinogen (normal 200-400 mg. per 100 cc.) is not significantly altered in disturbances of protein metabolism per se.

Increased Plasma Fibrinogen. (1) Conditions causing slight hepatic injury (hepatitis) and tissue destruction (inflammatory) are frequently associated with moderate increases in plasma fibrinogen.

(2) Most acute infections (except typhoid fever), septicemias, bacteraemias and particularly pneumococcal pneumonia, in which condition values up to 1000 mg. per 100 cc. may be observed.

(3) Pregnancy and menstruation (400-500 mg. per 100 cc.).

(4) Following x-ray irradiation.

(5) Focal infection (sinusitis, tonsillitis, cholecystitis).

(6) Nephrosis. In this condition the plasma fibrinogen may rise to 1000 mg. per cent, presumably in an attempt to compensate for the diminution in plasma albumin. This increase, as in the case of acute infections, is perhaps made possible by the existence of general increased cellular permeability, allowing the more ready entrance of the comparatively large fibrinogen molecule into the circulation.

(7) Increased fibrinogen values have been reported in multiple myeloma and lymphopathia venereum.

Decreased Plasma Fibrinogen. (1) Hepatic Insufficiency. Since fibrinogen is formed in the liver, conditions associated with diminution in hepatic function may result in a decreased concentration of plasma fibrinogen. This is particularly true of acute hepatic insufficiency such as is seen in severe arsphenamine hepatitis, chloroform, phosphorus and carbon tetrachloride poisoning and in acute yellow atrophy of the liver, in which condition extremely low values may be encountered. It is likewise decreased in some cases of cirrhosis of the liver.

(2) Typhoid Fever. The plasma fibrinogen is usually moderately diminished in typhoid fever, which is one of the very few infectious diseases in which this condition is observed.

(3) Temporary, following severe hemorrhage.

(4) Cachectic conditions, notably malignancy.

cedure, the following facts must be kept in mind: oxalated plasma may contain 0.3-0.4 Gm. per cent less protein than serum; the values for heparinized plasma may be as much as 0.65 Gm. higher than for oxalated plasma; blood must be collected with a minimum of stasis, since pressure intermediate between venous and arterial causes, in one minute, a protein increase of 0.17 Gm. per 100 cc, due to loss of water from the plasma.

These indirect methods, of determining plasma or serum protein concentration have proven to be very useful clinically, but they may give erroneous results under certain conditions. For example, there is evidence that in abnormal serum, as in glomerulonephritis, and in hepatic disease, and perhaps also in normal serum, the albumin as well as the globulin fractions are not homogeneous but contain molecules of varying sizes. The albumin and globulin fractions in the serum of patients with glomerulonephritis have been found to differ from those in normal serum.^{13,42,61,82} Consequently, discrepancies are often observed between the serum albumin or total protein concentrations and the tendency toward the development of edema, since there may be a decrease in the amount of smaller molecular species within the albumin fraction and a corresponding increase in larger molecular forms.⁸² This may result in a drop in colloid osmotic pressure of the plasma with no corresponding change in the relative proportions of albumin and globulin as determined by chemical procedures. It has also been found that the linear relationship between protein concentration and specific gravity of normal rabbit plasma does not hold for rabbits in a state of shock.²⁰ This detracts from the reliability of this procedure for the indirect estimation of the plasma protein concentration.

It has been amply demonstrated that depletion of plasma proteins from any cause results in edema and that the time of appearance and degree of edema depend more upon the concentration of albumin than upon that of globulin. It has been found by Moore and Van Slyke that patients with nephritis develop clinical edema (noncardiac) when the total plasma protein content falls below 5.5 ± 0.3 per cent or the albumin fraction below 2.5 ± 0.2 per cent. Experimentally, Barker and Kirk demonstrated that edema appears whenever the albumin fraction falls below 0.8 Gm. per 100 cc. and that it disappears promptly when the albumin level rises above 1 Gm. per 100 cc. With these figures the total plasma protein concentration is usually 4-4.5 Gm. per cent. It is also stated that edema usually appears when the plasma specific gravity falls below 1.023.⁸⁶ However, it seems obvious that no categorical statement can be

centration of albumin in the serum. Wicks and Peters believe that there is little basis for such an assumption and suggest the following formula: $P = 60.1A + 22.9G - 50.0$, in which P represents the colloid osmotic pressure of the plasma proteins in millimeters of water and A and G are expressed in terms of grams per 100 grams of water in serum or plasma. These authors believe that unless practicable methods for the measurement of serum colloid osmotic pressure can be greatly improved in accuracy, estimations from prediction equations such as the above may be quite as reliable, for relative purposes, at least, as direct measurements.

Keys suggests the following formula: $P = 45.2A + 18.8G$, where A is the albumin and G the globulin concentration in grams per 100 cc. By direct measurement, the colloid osmotic pressure of normal serum or plasma has been found to be 280-470 mm. H_2O (average 360 mm.), slightly lower values being obtained in normal pregnancy (average 300 mm.).

There is a linear relationship between the plasma specific gravity and total protein content,⁶⁴ normal heparinized plasma with 7 Gm. of protein per 100 cc. having a specific gravity of approximately 1.027 and an increase or decrease of 1 Gm. of protein per 100 cc. causing a rise or fall of 0.0029 in specific gravity. Increasing interest in alterations in the plasma protein concentration in clinical disorders accompanied by edema, dehydration and the shock syndrome has resulted in the common substitution of relatively simple methods for determining the plasma specific gravity for the more complicated and time-consuming chemical procedures. Inasmuch as albumin and globulin have about the same effect upon specific gravity, variations in the albumin:globulin ratio may be disregarded in this connection. Obviously, this procedure is of no value in distinguishing between changes in albumin and globulin as the cause of alterations in total protein content, and simultaneous decrease in one and increase in the other may be accompanied by little or no significant change in specific gravity.

Plasma specific gravity may be determined readily by the falling drop method,^{3,60} its relation to the protein concentration being expressed by the following formulae:⁶⁰

Serum Protein (Gm. per 100 cc.) = $345(G - 1.0076)$, where G is the specific gravity at 25° C.

Plasma Protein = $340(G - 1.0099)$.

The estimation of plasma protein is not quite as accurate as that of serum protein, but the accuracy probably satisfies clinical requirements. In interpreting results obtained by this pro-

made in this connection. Because of the operation of other complicating factors, such as abnormal variations in the size of the protein molecules and in capillary permeability, capillary blood pressure, lymph flow, tissue tension and salt and water intake, edema may be present at higher and absent at lower levels of protein and specific gravity in different subjects or in the same subject at different times (p. 257).

Diminution in plasma albumin, with compensatory increase in globulin and, at times, fibrinogen, and a decrease in or reversal of the albumin: globulin ratio but with a decreased total protein content occurs in the following conditions:

(1) **LOSS OF ALBUMIN.** This occurs most commonly, clinically, in nephritis and nephrosis with excessive and prolonged albuminuria.²³ In chronic nephritis as much as 60 Gm. of albumin may be eliminated daily in the urine and a daily loss of 4-5 Gm. may eventually result in a gradual decrease in the level of plasma protein. The plasma protein concentration usually remains within normal limits in the early stages of acute glomerulonephritis. Van Slyke has found that cases of this condition that maintain plasma albumin values above 2.2 Gm. per 100 cc. or total protein values above 5.5 Gm. per 100 cc. have a better prognosis than those which do not. Of his cases in the group with higher albumin values, only 13 per cent progressed into the chronic or terminal stages of nephritis, in contrast to 72 per cent of the group with low albumin values. It would appear, therefore, that the plasma protein concentration is of prognostic significance during the acute stage of glomerulonephritis. It was also found that a low plasma albumin concentration, usually below 2.5 Gm. per cent, was the rule in the chronic active stage of glomerulonephritis. As the chronic active stage progressed into the terminal stage, there was a marked tendency for the plasma proteins to increase, normal values being obtained in about half the cases during the terminal stage of the disease. This may be due in part to decreasing albuminuria and in part to hemoconcentration.

Extremely low values may occur in chronic nephrosis, falling at times to 0.2 Gm. per 100 cc. The existence of so-called "lipoid" nephrosis as a disease entity in adults is questionable, but it may occur in children (p. 400). Prolonged albuminuria in other forms of chronic nephrosis, amyloid disease of the kidneys and congestive heart failure may contribute to the development of hypoproteinemia. In nephrosis particularly, the globulin and fibrinogen are commonly markedly increased, the former rising to as high as 5 Gm. per 100 cc. and the latter to as high as 1 Gm. Decrease in albumin, with or without a decrease in

the total plasma protein concentration, occurs commonly in multiple myeloma; in some cases the globulin fraction is enormously increased, with consequent hyperproteinemia (p. 95).

It is probable that the blood plasma is the source of the albumin present in the urine although whether or not it represents normal blood protein is debatable. Similar changes have been produced experimentally by repeated plasmapheresis, with withdrawal of blood, separation of the plasma and reinjection of the corpuscular elements. It seems certain that the diminution in serum albumin which occurs in renal disease is not due entirely to loss of albumin in the urine. There appears to be a distinct nutritional defect which interferes with the ability of the organism to regenerate serum albumin. The importance of this factor is not sufficiently appreciated. It probably plays an important part in determining the development of hypoproteinemia in chronic glomerulonephritis. In the terminal stages of this condition the true extent of the serum albumin depletion may be masked by the development of a state of hemoconcentration, which may result from vomiting, anorexia and the inability to conserve the salt and water stores of the body. A diminution in serum albumin may occur in acute glomerulonephritis, particularly in the transition between this stage and the chronic active stage of the disease. Here it appears to be referable to loss of albumin in the urine, malnutrition and impairment of the ability to regenerate serum albumin.

In certain cases of ascites, the ascitic fluid containing a large amount of protein, depletion of the blood proteins may occur with changes similar to those observed in chronic nephritis with prolonged, excessive albuminuria. Edema may be present in such patients in the absence of renal or cardiac lesions. In advanced stages of chronic hepatic disease, including portal cirrhosis, there is also a moderate reduction in total serum protein, the diminution occurring chiefly in the albumin fraction.^{85, 108} In some cases, particularly in acute forms of liver disease, the serum albumin may be only moderately reduced and the serum globulin actually increased. The increase in globulin has been attributed to infection in such cases. Such findings are obtained much more commonly in primary hepatocellular disorders than in obstructive jaundice. The decrease in serum albumin in hepatic disease has been attributed by some chiefly to coexisting malnutrition, and by others chiefly to interference with regeneration of serum albumin resulting from impairment of liver function. In cases in which ascites is present, the loss of albumin from the blood plasma into the ascitic fluid probably plays some part in contributing to the plasma protein depletion.

Large amounts of albumin may be lost from the plasma also in extensive burns, severe hemorrhage and polyserositis (Pick's disease). Hypoproteinemia has been observed in shock due to burns, intestinal obstruction and generalized peritonitis, due presumably to loss of plasma from the circulation.³⁰

(2) **INADEQUATE SUPPLY OF PROTEIN.** This may occur in states of inanition, as in advanced tuberculosis and malignancy. It is observed in gastro-intestinal disorders accompanied by impaired protein digestion or absorption, prolonged vomiting or diarrhea. Prominent among such conditions are peptic ulcer, gastro-intestinal malignancy, pancreatic disease, intestinal fistula, short-circuiting operations on the intestine, chronic ulcerative colitis, tuberculous and other forms of chronic enteritis, congestive heart failure with edema of the gastro-intestinal mucous membrane, pellagra. This condition may also be associated with specific dietary deficiency characterized by low protein intake. This is well illustrated by the many instances of war edema, famine edema or nutritional edema which were observed during World War I. Cowie and Cooperstock demonstrated that edema could be induced at will in children suffering with chronic glomerulonephritis and nephrosis by varying the protein intake. When sufficient protein was administered to maintain a continuous positive nitrogen balance of +2 to +5 Gm., edema did not develop. Inadequate protein feeding resulted in the prompt development of edema with diminution in the plasma albumin concentration and reversal of the albumin:globulin ratio.

(3) **IMPAIRED SYNTHESIS.** The importance of this factor is not sufficiently appreciated. It probably plays an important part in maintaining the low level of plasma albumin in chronic hepatic disease, particularly cirrhosis, and in chronic glomerulonephritis; various infections, severe anemias (e.g., uncontrolled pernicious anemia), cachectic states and pregnancy and lactation. Intravenous injection of gum acacia is followed by impairment of plasma protein formation (hepatic malfunction?).

(4) **EXCESSIVE PROTEIN CATABOLISM.** Excessive breakdown of body protein, in conjunction with either inadequate supply or defective utilization, as in uncontrolled diabetes mellitus and severe thyrotoxicosis, may be accompanied by hypoproteinemia.

(5) **PREGNANCY AND LACTATION.** Several observers have reported a steady decrease in plasma protein concentration, due to diminution in the albumin fraction, during the course of normal pregnancy.^{81,109} Actually, subnormal values are rarely observed under normal conditions. It is believed by some, however, that an aggravation of this natural tendency induced by

dietary restriction of protein may lead to harmful consequences. Low values for plasma albumin and total protein have been reported in patients with toxemia of pregnancy, particularly eclampsia. The decrease in the concentration of plasma protein in normal pregnancy is attributed, in part at least, to the appreciably increased plasma hydration that occurs during that period (p. 514). However, it has been shown that not only the concentration but also the total amount of circulating plasma protein is diminished. This diminution is associated with marked impairment in the ability to regenerate serum protein. Melnick believes that synthesis of body proteins in the fetus constitutes a drain upon the maternal organism which is of primary importance in causing the lowered serum protein concentration characteristic of pregnancy. Similar findings have been obtained during lactation.

(6) PLASMA DILUTION. Hypoproteinemia may result from sudden dilution of the plasma which follows an acute, massive hemorrhage. A similar phenomenon may accompany sudden recovery from severe dehydration in malnourished subjects, as in diabetic coma and protracted vomiting or diarrhea (especially in children). In the dehydrated state, the actual plasma protein deficit may be masked by hemoconcentration (loss of plasma water). When the latter is corrected by administration of large quantities of solutions of sodium salts (chloride, bicarbonate, lactate, etc.), the plasma protein concentration may fall to a level sufficiently low to produce edema.

The frequently diminished plasma protein concentration in congestive heart failure is due in part to increased plasma volume (water). As indicated above, it is also contributed to in such cases by prolonged albuminuria, decreased protein intake, impaired digestion, diminished absorption from the edematous intestinal mucosa and impaired synthesis (hepatic malfunction).

Hyperproteinemia.^{8,55} An increase in the concentration of albumin in the plasma or serum is seldom encountered. It may occur in dehydration with diminution in the water content of the blood plasma and consequent increased concentration of all of its constituents in solution or suspension. In such conditions the globulin and albumin fractions will be proportionately increased. Although theoretically possible this condition is actually rarely observed, for in most instances in which dehydration is a prominent feature, complicating factors such as inanition, vomiting, diarrhea, etc., exert an influence which tends to decrease the plasma protein concentration. However, total protein values of 10-12 Gm. per 100 cc. have been reported in patients with cholera, other forms of severe diarrhea, partic-

ularly in children, shock, burns, diabetic acidosis, Addison's disease, intestinal obstruction, intestinal fistula, pyloric obstruction, marked restriction of fluid intake, certain fulminating infections and heat exhaustion.

Although a markedly increased concentration of total plasma protein is not observed commonly clinically, some degree of hyperproteinemia does occur rather constantly in several clinical disorders. In all of these the increase occurs only in the globulin fraction (including at times fibrinogen), the albumin fraction being almost invariably decreased. Frequently, the decrease in albumin suffices to offset the increase in globulin, the total protein concentration remaining within normal limits or decreasing.

There may be qualitative as well as quantitative changes in the serum globulins, as revealed by electrophoretic, chemical and immunological methods. The alpha-globulins are usually increased in febrile conditions. In chronic nephrosis ("lipoid"), there is an increase in alpha- and beta-globulins, whereas gamma-globulins are often diminished somewhat.¹⁹ In multiple myeloma, an increase has been found in gamma- and beta-globulins and, occasionally, in an abnormal globulin migrating between these two fractions. The electrophoretic patterns are normal in some cases.⁴⁷ Both alpha- and gamma-globulins are increased in tuberculosis. There seems to be a close correlation between the sedimentation rate and the alpha-globulin concentration, both increasing in the presence of considerable tissue destruction or inflammation.¹⁰⁸

The globulins have been separated chemically (precipitation by sodium sulfate) into three fractions, euglobulin (normal range 0.1-0.4 Gm. per 100 cc., pseudoglobulin I (0.8-1.9 Gm. per 100 cc.) and pseudoglobulin II (0.2-0.8 Gm. per 100 cc.). In cirrhosis of the liver and most chronic infectious diseases accompanied by hyperglobulinemia (syphilis, leprosy, rheumatic fever, rheumatoid arthritis, subacute bacterial endocarditis, tuberculosis, lymphopathia venereum, kala-azar, sarcoid, etc.), the increase is usually in the euglobulin and pseudoglobulin I fractions. The findings in multiple myeloma are indicated below.

Hyperglobulinemia, with or without actual hyperproteinemia, occurs commonly in the following conditions:

(1) Certain acute infections and acute and chronic suppurative conditions.⁸ These include pneumococcus pneumonia, subacute bacterial endocarditis, occasionally rheumatic fever and, at times, osteomyelitis and lung abscess. Total protein values as high as 10 Gm. per 100 cc. have been reported in subacute bacterial endocarditis, with globulin values ranging from 5 to

8 Gm. per 100 cc. There is evidence which suggests that the maintenance of a high plasma globulin concentration is responsible for the development of amyloid disease in conditions associated with chronic suppurative processes.

(2) Lymphopathia Venereum. This condition is rather consistently associated with hyperproteinemia. Gutman reported this finding in twenty-six of thirty-five cases, in ten of which the plasma protein concentration ranged from 9 to 11.2 Gm. per 100 cc. In all cases the albumin-globulin ratio was reversed, with a marked increase in the globulin fraction.

(3) Multiple Myeloma.^{17,26,32a,47,55,83} Hyperproteinemia appears to occur in 50-60 per cent of cases of multiple myeloma. Total protein values as high as 16 Gm. per 100 cc. have been reported, the average of twenty-one cases being 11.3 Gm. per cent. In this condition the increase occurs entirely in the globulin fraction. In some cases the increase in globulin is largely in the "euglobulin" fraction and partly in the "pseudoglobulin I" fraction (gamma-globulin by electrophoresis). In others, without hyperglobulinemia, evidence has been obtained suggesting the presence of Bence-Jones protein in the serum, while still others have yielded essentially normal findings.⁴⁷

(4) Boeck's Sarcoid.

(5) Miscellaneous conditions. Elevation of the total plasma protein concentration with reversal of the albumin-globulin ratio, and characterized especially by marked increase in globulin, has been reported in malaria, myeloid, monocytic and lymphatic leukemia, syphilis, rheumatoid arthritis, filariasis, tuberculosis, trypanosomiasis, lupus erythematosus, periarteritis nodosa, cirrhosis of the liver, leprosy, schistosomiasis and kala-azar. However, although an increase in plasma globulin is not uncommonly observed in these conditions, the total plasma protein concentration is usually within normal limits.

Abnormal Globulin Reactions^{8,55,65}

Several procedures have been proposed for the diagnosis of certain conditions, the majority of which depend chiefly upon the presence of an increase or some qualitative change in one or more of the plasma globulin fractions.

Takata-Ara Reaction.^{77a,118a} This reaction is believed to be dependent upon the following phenomenon: mercuric chloride and sodium carbonate form a colloidal solution of mercuric oxide in the presence of normal plasma proteins in normal concentration. In certain pathologic states there is precipitation of the mercuric oxide sol, apparently due in many cases at least to increase or qualitative abnormality in the globulin fraction.

Positive reactions have been obtained in the majority of cases of hepatic cirrhosis and in several other forms of severe hepatocellular damage. A negative reaction may be useful at times in differentiating such conditions from carcinoma of the liver or obstructive types of jaundice, but the fact that positive reactions may occur in the latter and negative reactions in the former seriously impairs the diagnostic value of this procedure. Positive reactions have been obtained in several conditions other than hepatic disease in which the serum globulins are abnormal. These include pneumonia, syphilis, tuberculosis, acute glomerulonephritis, the nephrotic syndrome and multiple myeloma. The reaction has been advocated widely as of value in the diagnosis of cirrhosis of the liver, in which condition positive findings are obtained most consistently, but its usefulness is questionable. The specific relation of hyperglobulinemia to the production of this reaction has not been definitely established.

Formol-gel Reaction.^{7,43} The addition of formaldehyde to normal serum, under standardized conditions, results in no change in viscosity or translucency. Positive reactions (abnormal) are indicated by increased viscosity (gelation), with or without increased opacity. A positive reaction is commonly obtained in conditions accompanied by significant hyperglobulinemia, but may also occur in other conditions, the exact mechanism of its production being still questionable.

Positive reactions have been reported in multiple myeloma, subacute bacterial endocarditis, Boeck's sarcoid, hepatic cirrhosis and other forms of hepatic parenchymal disease, chronic glomerulonephritis, malaria, trypanosomiasis, kala-azar, schistosomiasis and a variety of other acute and chronic infectious diseases. A strongly positive reaction in rheumatic fever has been reported to be suggestive of the presence of rheumatic carditis.¹⁰⁰

Colloidal Gold Curve.⁵⁹ A paretic type of colloidal gold curve has been obtained with the blood serum of a considerable proportion of patients with various forms of liver disease (cirrhosis, necrosis, hepatitis, malignancy), due apparently to some qualitative or quantitative change in the euglobulin or gamma-globulin fraction.⁴⁵

CO₂ Saturation Test.^{85a} In the presence of globulin concentrations over 3-4 per cent, the addition of distilled water to the serum causes clouding due to flocculation of euglobulin. This reaction can be accelerated by saturating the diluted serum with CO₂ and by lowering the pH toward the isoelectric point of globulin. Positive (abnormal flocculation) reactions have been obtained in conditions accompanied by serum globulin concentrations above 3 Gm. per 100 cc. and in some cases with

normal serum globulin concentrations. In the latter, the reaction may be dependent upon an increase in the euglobulin fraction. Results obtained with this procedure are approximately the same as with the Takata-Ara and formol-gel reactions.

Cephalin-cholesterol Flocculation Test.^{50,58,92,96} This test gives positive results (flocculation) in a large proportion of cases of active hepatocellular damage and is widely used in the study of patients with jaundice (p. 424). Positive findings are also obtained in most of the other conditions mentioned above in which there is an increase in globulin, especially if the albumin is decreased. The mechanism of production of cephalin-cholesterol flocculation in pathologic serum is not entirely clear, but it has been demonstrated that increase in gamma-globulin can produce this reaction.⁵⁸ Positive reactions may be obtained with normal serum after standing at ice-box temperatures for several months or after heating to 56° C. for thirty minutes.⁵⁸

Other Globulin Reactions. The Weltmann reaction is based upon the fact that a 1:50 dilution of serum does not coagulate on boiling, but will do so when a minimal amount of calcium chloride is added. With normal serum the concentration of added calcium chloride must be at least 0.35:1000, while coagulation may occur with concentrations as low as 0.2:1000 in serum from patients with conditions mentioned in connection with the reactions discussed above. This phenomenon apparently depends upon the presence of an increase in euglobulin or a qualitative change in the globulin fraction, as does the magnesium flocculation reaction (Bauer).⁴ The latter may be positive also in pernicious anemia.

Abnormal Proteins in Blood

Hemoglobin. Hemoglobinemia (hemoglobin free in the plasma) may occur when excessive numbers of erythrocytes are destroyed very rapidly, particularly when the hemolytic process occurs intravascularly. The condition is accompanied by anemia, reticulocytosis, an increase in serum bilirubin and, at times, methemoglobinemia and methemalbuminemia. The latter pigment is formed from hematin by combination with plasma albumin.³¹ If the concentration of hemoglobin in the plasma exceeds 60-150 mg. per 100 cc., hemoglobinuria may occur (p. 119), and may persist until the plasma Hb has fallen to 30-50 mg. per 100 cc.^{40,41} Excessive amounts of urobilinogen are excreted in the urine and feces (pp. 445, 447)

Hemoglobinemia therefore is a manifestation of severe hemolytic anemias, especially the acute variety, but also ex-

accrations of chronic forms. The following factors may be active in bringing about this condition:

(a) *Bacteria*. Hemolytic streptococcemia, yellow fever, severe typhoid and scarlet fevers, clostridia infections.

(b) *Protozoa*. Malaria and blackwater fever and Bartonella infection (Oroya fever).

(c) *Chemical Agents*. Poisoning with arseniuretted hydrogen, arsphenamines, pyrogallol acid, phenylhydrazine, saponin, sulfonamides and, rarely, lead. Some unchanged hemoglobin may appear in the plasma at times in poisoning with substances that cause methemoglobinemia.

(d) *Vegetable Poisons*. Poisoning with ricin, crotin and poisonous toadstools; favism, due to hypersensitivity to the fava bean.

(e) *Animal and Endogenous Poisons*. Poisoning with certain snake venoms and occasionally after severe, extensive burns, administration of serum for tetanus, diphtheria and meningococcus meningitis.

(f) *Miscellaneous*.¹²⁴ Transfusion with incompatible blood, paroxysmal hemoglobinuria (cold hemolysins and syphilis), paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli),^{35, 42, 125} acute hemolytic anemia of unknown etiology (Lederer's anemia),²⁴ acute crises in congenital hemolytic jaundice (spherocytic anemia),³⁸ march hemoglobinuria (strenuous exertion, lordosis),⁴⁰ and occasionally after severe intra-abdominal hemorrhage.

Methemoglobin. This is an oxide of hemoglobin (oxidized heme), differing from oxyhemoglobin (oxygenated hemoglobin) in that it contains ferric iron in contrast to ferrous iron in the latter and that its oxygen is in firm combination and cannot be removed by exposing the blood to a vacuum. Methemoglobin is consequently incapable of functioning as an oxygen-carrier (p. 301). Cyanosis usually develops when the concentration reaches 3 Gm. per 100 cc. of blood.

Methemoglobin is usually formed within the red blood cells, which generally remain intact. In severe cases, however, the cells may be injured and destroyed and methemoglobin, often with hemoglobin, may be liberated into the plasma and be excreted in the urine. The causes of methemoglobinemia may be classified under four headings:

(a) *Drugs and Other Toxic Agents*. These include nitrites, chlorates, permanganates, aniline, acetanilid, acetphenetidin, antipyrine, nitrobenzene, pyrogallol, sulfonal, plasmochin, methylene blue and certain sulfonamides.^{51, 122}

(b) *Other Hemolytic Agents.* Among conditions falling under this heading are congenital hemolytic icterus (acute crises), paroxysmal hemoglobinuria, infection with anaerobic bacteria (clostridia), blackwater fever and excessive doses of phenylhydrazine.

(c) *Enterogenous Cyanosis.* In some cases so classified the production of methemoglobinemia is believed to be due to excessive production and absorption of nitrites from the intestine. These nitrites may be elaborated by nitrite-producing bacteria.

(d) *Idiopathic.* This is an unusual condition in which cyanosis (methemoglobinemia) is constant and unaffected by therapy. Such cases may be confused with congenital heart disease.

Sulfhemoglobin. Reduced hemoglobin combines with hydrogen sulfide to form sulfhemoglobin. Sulfhemoglobinemia is an unusual condition which occurs chiefly as a result of the action of nitrites and coal tar preparations (aniline, acetphenetidin, acetanilid) in the presence of excessive amounts of sulfur. It has also been observed in subjects with marked constipation, in the presence of nitrite-producing bacteria in the intestine. Some believe that aniline may "sensitize" hemoglobin so that it unites with sulfides. This condition has been produced in splenectomized animals by feeding large amounts of sulfur without additional medication. Sulfhemoglobinemia has been reported in patients receiving sulfonamides.⁵¹ As is the case in methemoglobinemia, sulfhemoglobin is usually contained in the erythrocytes, but it may occasionally be liberated into the plasma. Cyanosis usually occurs when the concentration of sulfhemoglobin reaches 3-5 Gm. per 100 cc. of blood.

Carboxyhemoglobin (p. 301). Carbon monoxide combines with the same group in the hemoglobin molecule as does oxygen, the combining capacity of hemoglobin for both being identical. However, the affinity for carbon monoxide is more than 200 times as great as for oxygen, and in the presence of relatively small concentrations of CO in the air a considerable quantity is taken up by the blood, with a consequent reduction in the amount of hemoglobin available for transport of oxygen to the tissues. The dissociation of oxyhemoglobin is also diminished. Carboxyhemoglobin has a bright, cherry-red color.

Other Abnormal Proteins. Qualitative abnormalities have been demonstrated in the plasma albumin and globulins in certain disease states. Immunological differences have been reported between the albumin and globulin fractions of normal subjects and patients with hepatic disease and chronic glomerulonephritis and nephrosis.^{42,43,61} Abnormalities of the cystine and

tyrosine content of the plasma proteins have been observed in chronic glomerulonephritis.¹¹⁶ Osmotic pressure studies suggest that in patients with chronic nephritis (nephrotic stage) the molecular weights of the plasma albumin and globulin are nearly double those of normal albumin and globulin, while the molecular weights of the urine proteins are lower than normal. These findings suggest that plasma albumin and globulin are heterogeneous fractions containing molecules of different sizes, the smaller passing more abundantly through the glomerular membrane into the urine.^{13,42} Other evidence of abnormality in the plasma protein fractions has been referred to elsewhere (p. 89).

Bence-Jones protein has been demonstrated in the blood serum in a few cases of multiple myeloma, but this finding is by no means common.^{17,49,43}

NONPROTEIN NITROGEN OF BLOOD

Alterations in the total nonprotein nitrogen content of the blood depend upon variations in the concentrations of its constituent elements, which will be considered individually (see also Renal Function, p. 366).

Urea Nitrogen (normal 9-17 mg. per 100 cc.). *Increased Urea N* (see Renal Function, pp. 362, 371). Increase in blood urea *N* may be due to (a) decreased renal excretion, (b) absorption from the intestine of excessive amounts of products of protein digestion or (c) excessive protein catabolism, or to a combination of these factors. Elevation of blood *NPN* or urea *N* due primarily to factors other than renal or urinary tract disease has been termed "extrarenal azotemia." Decreased renal excretion of urea may be due to (a) organic disease of the kidneys, with destruction of a considerable portion of functioning renal tissue or (b) abnormality of the mechanism of glomerular filtration, resulting in significant decrease in the effective filtration pressure (p. 342) (e.g., marked hypotension, increased capsular pressure, hemoconcentration, hyperproteinemia). The most common cause for increased blood urea *N* (and *NPN*) is inadequate excretion, due usually to kidney disease, urinary obstruction or extreme hypotension and oliguria.

(1) **GLOMERULONEPHRITIS.** This is a common cause for abnormally high blood urea *N* concentrations, evidence of renal functional impairment in acute and chronic glomerulonephritis. In the terminal stage and during acute exacerbations of chronic nephritis, values as high as 200 mg. or more per 100 cc. may be obtained (p. 368).

(2) **OTHER KIDNEY DISEASES.** These include (a) conditions in which there is extensive destruction or inflammation of the

kidneys, as in renal tuberculosis, pyelonephritis, advanced nephrosclerosis, renal cortical necrosis, malignancy, suppuration and chronic gout; (b) renal conditions accompanied by marked oliguria or anuria, as bichloride of mercury poisoning, post-operative urinary suppression and advanced myocardial failure with passive congestion of the kidneys; (c) congenital renal lesions, as hypogenesis or hypoplasia and polycystic disease of the kidneys; (d) conditions in which the uriniferous tubules are blocked by precipitation of some substance which interferes with urine excretion. Among these are multiple myeloma, amyloid disease of the kidneys, hemoglobinuria, as after transfusion with incompatible blood and sulfonamide therapy.

(3) URINARY TRACT OBSTRUCTION. Enlarged prostate, urinary lithiasis, malignant obstruction of the ureters from extension of tumors of the bladder or uterus, bowel, retroperitoneal lymph nodes.

(4) HEPATIC AND BILIARY TRACT DISEASE. High *NPN* and urea *N* values are encountered at times in patients with hepatic disease. This occurs most commonly in terminal stages of acute or chronic liver disorders, after operation on the bile passages, particularly after decompression of obstructed bile ducts, and in traumatic necrosis of the liver. The mechanism operating in such cases to produce the so-called "hepato-renal syndrome" is not clearly understood.

(5) SHOCK AND HEMOCONCENTRATION. Hemoconcentration resulting from loss of plasma water by prolonged vomiting, diarrhea or sweating may result in elevation of blood urea, especially if accompanied by severe hypotension, as in the shock syndrome. These phenomena prevent adequate glomerular filtration by diminishing the effective glomerular filtration pressure and also result in extreme oliguria by prerenal deviation of water. In this category may be placed severe burns, high intestinal obstruction, pyloric obstruction, cholera, typhoid fever, infantile diarrhea, dysentery, Addison's disease, pancreatic fistula, peritonitis.

(6) EXCESSIVE PROTEIN CATABOLISM. Slight or moderate increase in blood urea may occur in severe toxic and febrile conditions in which tissue protein catabolism is accelerated and in which there is also some degree of renal functional impairment. This factor may contribute to the increase in blood urea in many of the conditions mentioned above (acute intestinal obstruction, severe infections and burns).

(7) MISCELLANEOUS. Nitrogen retention may occur as a consequence of renal failure, usually as a terminal event, in patients with hyperparathyroidism (p. 177). This apparently

depends in part upon extensive calcification of the renal tubular epithelium or upon the formation of urinary calculi; in acute hyperparathyroidism, hemoconcentration and impaired blood flow through the kidneys may contribute to the development of renal functional insufficiency.

Nitrogen retention is observed not infrequently in diabetic coma. The mechanism of its production is not clearly understood. However, dehydration, hemoconcentration, excessive protein catabolism and perhaps actual renal damage may be of importance in this connection.

The blood urea may rise soon after hemorrhage into the gastrointestinal tract (peptic ulcer, carcinoma of the stomach, esophageal varices in cirrhosis of the liver, and so on) due probably to absorption of excessive amounts of products of protein digestion (from globin and albumin) and inadequate renal excretion.^{18, 57, 57, 126}

Decreased Urea N. (1) ACUTE HEPATIC INSUFFICIENCY. As urea is normally formed in the liver and, presumably, through hepatic functional activity, it naturally follows that a decrease in blood urea may be expected in conditions associated with acute hepatic insufficiency. Subnormal values (5-10 mg. per 100 cc.) have been observed in acute yellow atrophy of the liver, acute toxic hepatic necrosis due to phosphorus, arsphenamine, chloroform and carbon tetrachloride poisoning, eclampsia and in acute hepatic insufficiency following operative procedures upon the biliary tract. Such findings are rare in chronic hepatic disease such as cirrhosis, passive congestion or malignancy because of the large functional reserve capacity and enormous regenerative power of the liver.

(2) NORMAL PREGNANCY. The blood nonprotein nitrogen decreases during the first six months of pregnancy to levels of 20-25 mg. per 100 cc., increasing subsequently to reach an average of 25-30 mg. per 100 cc. at term. The urea nitrogen may fall as low as 5 mg. per 100 cc. after six months, these low levels being maintained until the eighth or ninth month, when it begins to rise, reaching values of 7-9 mg. per cent at term.

Uric Acid (normal 2-4 mg. per 100 cc.). Abnormal elevation of the uric acid content of the blood may occur as a result of (1) diminished uric acid elimination, (2) primary alteration in nucleoprotein metabolism (gout), (3) increased endogenous nucleoprotein catabolism, (4) excessive nucleoprotein or purine supply in individuals in whom uric acid elimination or purine metabolism are disturbed, and, questionably in man, (5) diminished uric acid destruction. The blood uric acid concentration is at times subnormal in pernicious anemia during relapse.

(1) *Renal Functional Impairment.* Increased values are observed in both acute and chronic nephritis with nitrogen retention. Retention of uric acid in the blood frequently, although not invariably, precedes that of urea and creatinine. Values of 4-10 mg. per 100 cc. are commonly observed; figures above 15 mg. per 100 cc. are unusual. The administration of purine-rich foods to such persons may be followed by an increase in the concentration of uric acid in the blood. This does not occur in normal individuals.

In conditions associated with urinary obstruction (prostatic, bilateral ureteral calculus, etc.), urinary suppression or renal destruction (tuberculosis, pyelonephritis, hydronephrosis, polycystic kidneys, etc.), in renal hypogenesis and congestive heart failure, uric acid may be retained in association with other urinary constituents.

(2) *Gout.* Gout is a primary disorder of nucleoprotein or purine metabolism, the exact nature of the metabolic error being unknown. Chemically this condition is characterized by a rise in the resting level of circulating (and, perhaps, tissue) uric acid, which may increase immediately before and during acute attacks to 6-10 mg. per 100 cc. or higher. In the diagnosis of gout, uric acid determinations should be made in serum or plasma collected under anaerobic conditions.⁵⁴ Hyperuricemia is an almost invariable feature of acute gout, serum uric acid values below 6 mg. per 100 cc. being seldom encountered, and figures as high as 15 mg. per 100 cc. being obtained at times. There is, in uncomplicated cases, no associated increase in blood urea or creatinine. In chronic gout, nephritis is a common complication and eventually retention of these substances occurs. The ingestion of purine-rich foods is followed by an increase in blood uric acid and may precipitate an acute attack.

Many authors believe that the fault lies in the intermediary metabolism of purines rather than primarily in the kidney. Minkowski held that uric acid exists in the blood in organic combination, probably as a nucleotide or nucleoside which is readily dissociable, the uric acid being normally removed from this combination and excreted by the kidneys. Severin advanced the hypothesis that, in gout, owing perhaps to defective deamination of purines by the liver, this uric acid combination is so altered that it cannot be readily dissociated and eliminated by the kidneys. Consequently it is retained in the blood at an abnormally high level and is deposited particularly in cartilage, which appears to have a specific affinity for urates. However, more recent studies have failed to reveal evidence of abnormal intermediary metabolism of purines in patients with gout.

Some observers^{11a} suggest that the increase in blood uric acid in gout may be due to increased production, but there is no evidence of accelerated nuclear metabolism in this condition.⁵

Other investigators are of the opinion that the kidney is primarily at fault. It has long been recognized that in most mammals uric acid may be destroyed, with the production of allantoin; there is available, however, no direct evidence of a similar process in man. Folin and his associates injected uric acid (lithium urate) into the blood stream of normal men and found that only from 30 to 90 per cent (average 50 per cent) was eliminated in the urine in from one to four days and that no uric acid could be demonstrated in the tissues. They believe that 10 to 70 per cent is destroyed in the body. In animals (dogs, cats, goats, rabbits) the injected uric acid is largely removed by and retained chiefly in the kidneys, the remainder being rapidly destroyed in the blood stream, only a relatively small proportion appearing in the urine. The portion stored in the kidneys is gradually returned to the blood and, in large measure, destroyed. Folin believes that normally the mechanism is essentially the same in man as in other mammals, a quantitative difference existing however, in that the human kidneys are less sensitive to uric acid and consequently uric acid destruction is more active and its concentration in the blood is higher. He states that the intermediary purine metabolism in gout is essentially normal and that the fault lies in a diminished responsiveness of the kidneys resulting in diminished elimination of uric acid from the blood; the level of circulating uric acid is therefore raised. However, the absence of direct proof that uric acid is destroyed in the human organism renders this hypothesis untenable at the present time.

Tests of renal function, such as the urea and inulin clearance tests, concentration tests and phenolsulfonphthalein excretion, may be normal in subjects with gout with hyperuricemia, but there is some evidence of a specific impairment of uric acid excretion.^{5,16} In subjects with hyperuricemia due to leukemia or resolving pneumonia, the concentration of uric acid in the urine is fifteen to thirty times that in the blood, but urine uric acid concentrations of this magnitude do not occur in gout with even higher levels of blood uric acid. Whereas the urate clearance of normal subjects is increased considerably if the blood uric acid is increased, this does not occur in subjects with gout, in whom the urate clearance (at high blood urate levels) is essentially the same as in normal subjects with normal blood urate concentrations. Under such circumstances, about 90 per cent

of the urate filtered through the glomeruli is reabsorbed in the tubules.^{21,22} These observations indicate that the urate clearance in gouty subjects with hyperuricemia is lower than in nongouty subjects, provided the blood uric acid concentration and the rate of urine excretion are comparable.⁵

(3) *Leukemia*. An increase in blood uric acid occurs commonly in chronic leukemia, particularly myelogenous leukemia. This is, in all probability, a result of the active and greatly increased endogenous nucleoprotein metabolism (cell nuclei) which is present in that condition. A similar mechanism may operate to produce an increase in some cases of multiple myeloma, polycythemia vera and resolving pneumonia.

(4) *Remissions in Pernicious Anemia*. Riddle has demonstrated the relationship between the increased activity of the hematopoietic system during remissions in pernicious anemia and the endogenous uric acid metabolism. The earliest recognizable change is an increase in blood uric acid, due to increased endogenous purine metabolism, which precedes, by twenty-four hours or more, the increase in reticulocytes. This occurs during spontaneous remissions as well as in those induced by the administration of liver or liver extract. The curve of uric acid parallels that of reticulocytes. It is believed that, as stated by Riddle, "The great numbers of red cell nuclei, lost during the maturation process as red blood cells in the bone marrow assume their non-nucleated form, may be an important source of the increased amounts of uric acid found in the blood and urine during early remission . . . A generalized increase of nuclear metabolism throughout the body during remission seems worth mentioning as another factor possibly of importance."

(5) *Osteoarthritis*. Elevation in blood uric acid has been reported in patients with arthritis not associated with gout. In such cases, however, it is extremely difficult to exclude early chronic nephritis as the real cause of uric acid retention.

(6) *Chronic Lead Poisoning*. High values are frequently observed in chronic plumbism. Here, too, renal damage may be an important factor

(7) *Eclampsia*. Increase in uric acid occurs commonly in eclampsia and is sometimes attributed to increased endogenous nucleoprotein metabolism. It occurs without associated changes in creatinine or urea but mild grades of renal insufficiency may nevertheless be responsible. A slight increase may occur in normal pregnancy.

(8) *Intestinal Obstruction* (sec p. 101). Retention of all of the nonprotein nitrogenous constituents of the blood, including uric acid, may be observed in acute intestinal obstruction.

(9) *Hepatic Functional Impairment.* An increase in the uric acid concentration of the blood has been observed experimentally in animals following total hepatectomy. However, significant alterations in the blood uric acid concentration are seldom observed clinically in hepatic disease, although elevated values have been reported occasionally in patients with severe acute liver damage, as in acute and subacute hepatic necrosis such as occur in cases of chloroform and carbon tetrachloride poisoning.

Creatinine (normal—1–2 mg. per 100 cc.). An increased concentration of creatinine may occur in any condition in which blood urea is increased, but usually only after the latter has risen to comparatively high levels. This is due in part perhaps to extrarenal causes, particularly the solely endogenous origin of creatinine as contrasted to the largely exogenous origin of urea, and also to the fact that the normal creatinine clearance is considerably higher than the urea clearance (pp. 355, 372).

(1) *Nephritis* (see Renal Function, pp. 365). When renal functional impairment is due to chronic nephritis, creatinine retention is of decidedly serious prognostic import, since the nature of the anatomic lesion renders functional improvement impossible. In chronic nephritis with uremia, figures as high as 35 mg. per 100 cc. may be obtained. In this condition, values above 5 mg. per 100 cc. usually indicate a hopeless prognosis, with a relatively short expectation of life. In acute nephritis, however, and in acute exacerbations of chronic nephritis, extremely high concentrations of creatinine may be found, which, with subsidence of the acute renal lesion, may return to normal (see Renal Function, pp. 367).

(2) *Urinary Obstruction.* Prostatic and bilateral ureteral obstruction may, if the degree of back pressure rises sufficiently, be associated with creatinine, as well as urea and uric acid retention. Similar findings may be observed in unilateral ureteral calculus with reflex anuria. Under such circumstances, the process being purely mechanical in the absence of renal damage, the degree of elevation of blood creatinine is roughly proportional to that of urea; this is in striking contrast to the findings in chronic nephritis, in which condition high urea values may be observed in association with normal creatinine figures. In urinary obstruction, as in acute nephritis, in the absence of irreparable renal damage, extremely high creatinine values (15–30 mg. per 100 cc.) may return to the normal level following relief of the obstruction.

(3) *Urinary Suppression* (see Urea, p. 100).

(4) *Cardiac Decompensation* (see Urea, p. 101).

(5) *Intestinal Obstruction* (see Urea, p. 101).

Amino Acid Nitrogen (normal, 5-8 mg. per 100 cc.). Increase in the amino acid content of the blood may occur in the following conditions:

(1) *Hepatic Insufficiency*. Amino acids normally undergo deamination in the liver with the consequent formation of urea. When the urea forming or deaminizing function of the liver is impaired the concentration of amino acids in the blood increases and that of urea diminishes. The normal ratio of urea nitrogen to amino acid nitrogen is approximately 2 to 1. This ratio is, therefore, decreased in such conditions as acute yellow atrophy (acute toxic necrosis) of the liver, phosphorus, arsenic, chloroform and carbon tetrachloride poisoning and, at times, in acute catarrhal jaundice. Figures as high as 200 mg. per 100 cc. have been reported in cases of acute yellow atrophy of the liver but the usual range is 10 to 15 mg. per cent, values above 30 mg. being infrequently observed. The increase in amino acid nitrogen is due partly to extensive autolysis of hepatic tissue. High values are not ordinarily found in chronic hepatic disease such as cirrhosis, chronic obstructive jaundice, hepatic syphilis or malignancy because of the large functional reserve and marked regenerative power of the liver.

(2) *Eclampsia*. Figures of 6-12 mg. per 100 cc. are at times observed in eclampsia, due presumably to the hepatic lesions present in that condition.

(3) *Interference with Elimination*. The blood amino acid nitrogen may be found to be increased slightly in some cases of advanced nephritis with marked nitrogen retention and also in urinary obstruction or suppression. High values are, however, rare under these circumstances, unless there is an associated condition of hepatic insufficiency. In most cases of nephritis with high blood nitrogen values the amino acid content of the blood is within normal limits.

(4) *Miscellaneous*. An increase may occur occasionally in myeloid leukemia and, rarely, in diabetes mellitus, acute infections, congestive heart failure, hyperthyroidism and severe anemia.⁹⁰

Subnormal levels have been reported in pneumococcic pneumonia³² and after administration of anterior pituitary extracts (growth principle), due apparently to increased protein anabolism and decreased catabolism.^{87, 103}

Undetermined or Residual Nitrogen (normal, 5-18 mg. per 100 cc.). The undetermined or "residual" nitrogen is contained chiefly in the corpuscles and is believed by some to be in the form of hippuric acid, nucleotides and histones. In some cases

of eclampsia and advanced chronic nephritis with nitrogen retention and uremia the undetermined nitrogen fraction may be increased much more, proportionately, than the known nitrogenous elements. Some investigators believe that some component of this fraction may be responsible for the toxic manifestations of eclampsia and uremia. In some instances, however, no increase is demonstrable. A slight increase may occur in normal pregnancy.

Total Nonprotein Nitrogen (normal, 25-35 mg. per 100 cc. See Renal Function, pp. 366-371). An increase in the concentration of total nonprotein nitrogen occurs particularly in those conditions in which the blood urea content is increased. Extremely high values (to 400 mg. per 100 cc.) have been observed in chronic nephritis, acute nephritis, urinary obstruction and renal destruction (tuberculosis, pyonephrosis, polycystic kidneys, etc.). Slight elevations may be found in gout and in myelogenous leukemia, in which conditions the uric acid concentration is increased independently of urea and creatinine. Normal values are usually present in eclampsia.

ABNORMAL URINARY NITROGEN

Protein in Urine. Under normal conditions very little or no protein is eliminated in the urine. The uriniferous tubules of the kidney may be collectively regarded as a semipermeable membrane, existing as a barrier between the blood plasma and the urine, allowing the passage of water and most crystalloids, but being practically impermeable to colloids. By utilizing extremely delicate reagents such as an acid alcoholic solution of phosphotungstic acid (Tsuchiya reagent) and by Kjeldahl determinations, small amounts of protein may be demonstrated in the urine of many normal individuals. Addis has placed the upper limit of normal variation in the neighborhood of 30 mg. of protein for the twelve-hour-day period but values as high as 75 mg. daily may be normal. This consists almost entirely of albumin with a small proportion of globulin; these are in all probability derived from the plasma proteins. This normal proteinuria cannot be detected by the qualitative methods most commonly employed clinically.

The Nature and Origin of Urinary Proteins. The term "albuminuria" has become so deeply rooted in medical literature that it seems advisable to point out the fact that proteins other than albumin may be and commonly are present in the urine. In fact, albumin seldom appears in the urine in any quantity without the simultaneous appearance of some other protein, notably globulin. Consequently the term "proteinuria" is more

exact and more desirable than "albuminuria." Because of the universal acceptance of the latter term, however, it will be employed throughout the subsequent discussion to designate that group of protein substances which respond to the commonly employed qualitative and quantitative tests for albumin (heat and acetic acid, nitric acid, sulfosalicylic acid, and so on). Only on rare occasions is fibrinogen present in the urine in renal disease.

The bulk of accumulated evidence points to the fact that the greater part of the protein found in the urine in renal disease is derived from the plasma proteins, albumin, pseudoglobulin, euglobulin and fibrinogen. The proportion of each of these substances eliminated by the kidney in nephritis appears to be determined by the relative size and viscosity of their molecules. Therefore albumin, having by far the lowest molecular weight and viscosity, is excreted in largest amount and fibrinogen, possessing the largest molecule, is eliminated in smallest quantity, pseudoglobulin and euglobulin occupying intermediate positions in the order named.

The relative proportions of albumin and globulin present in the urine in various types of renal disease have been extensively investigated. Although the determination of the urinary albumin:globulin ratio has not proved to be of practical clinical significance, certain observations are of interest inasmuch as they illustrate to some extent the nature of the renal functional disturbance in the presence of different renal lesions. Extremely high urinary albumin:globulin ratios have been found in lipoid nephrosis, figures above 10 being commonly observed. In chronic glomerulonephritis the ratio is usually lower, the majority ranging from 3 to 5 with a few between 5 and 10. In acute nephritis the figures are usually low (4-6), rising as the acute process subsides. Low values are obtained in amyloid disease of the kidneys (0.5-3.5). Since the plasma albumin:globulin ratio is normally 1.5-2.5:1 and since the passage of various protein molecules through capillary walls and other living membranes is dependent upon increased permeability of those membranes, it appears that in amyloid disease the permeability of the kidneys is so increased that the relatively large globulin molecule passes freely, resulting in a urinary albumin:globulin ratio which approaches that present at the time in the blood plasma. Likewise, in acute nephritis, renal and capillary membrane permeability is so increased that globulin passes with relative ease; as the acute lesion subsides permeability diminishes and the ratio increases correspondingly. Finally, in lipoid nephrosis, membrane permeability being relatively

low, only small amounts of globulin are eliminated whereas the comparatively small albumin molecule passes through in large amounts.

The preceding brief discussion of the mechanism of proteinuria is based upon the conception that the bulk of the urinary proteins consists of normal plasma proteins and that their appearance in the urine is determined by varying degrees of increased renal membrane permeability. This concept is in accord with most experimental observations but a certain amount of evidence has been advanced which suggests that the fault may not lie entirely in the glomerular filter, and that the plasma proteins may be primarily altered in such a manner as to render their elimination from the body not only possible but necessary. It is well recognized that, whereas normal plasma protein is retained in the blood, foreign protein entering the blood plasma is readily eliminated by the kidney. Thus hemoglobin, when free in the plasma, and egg albumin and other foreign proteins, if introduced into the blood stream, are promptly eliminated in the urine. Whether or not proteinuria produced in this manner is dependent upon renal injury has not been definitely established, although the observation of Ascoli that there is usually an associated excretion of native plasma protein would seem to indicate that such is the case. Some observers, however, adhere to the so-called "humoral" origin of albuminuria. Among the more recent of these is Epstein, who believes that lipoid nephrosis is primarily a disturbance of protein metabolism as a result of which albumin is lost in the urine. He accordingly proposes the term "diabetes albuminuricus" as more appropriate and less misleading than "nephrosis." Welker, Andrews and Thomas reported the finding of small amounts of highly dispersed toxic protein in the urine of nephritic patients. They found that whereas, when normal serum is dialyzed against distilled water no appreciable amount of protein passes through the membrane, if the serum of nephritic patients or of animals with experimentally induced uremia is employed, proteins appear in the dialysate. These investigators believe that the serum proteins in nephritis and uremia combine with other nitrogenous fractions of tissue origin (perhaps hepatic), some of which are highly toxic. This combination is in the nature of a detoxicating process; the combination of normal serum proteins with these split products constitutes a substance which is foreign to the circulation and which is consequently eliminated by the kidneys. In apparent confirmation of this view is the observation that the urine in nephritis contains a relatively nontoxic peptone combined with or adsorbed by

serum proteins and also a highly toxic blood protein so highly dispersed in solution as to pass through a collodion membrane.

There is also evidence that the plasma proteins in glomerulonephritis and nephrosis differ from those in normal individuals in regard to their cystine and tyrosine content.^{1,15} Goettsch and Reeves have reported data indicating that during the edematous stage of nephrosis the serum proteins differ immunologically from normal serum proteins in that both albumin and globulin fail to precipitate completely with antisera developed against normal serum albumin and globulins. During convalescence, after edema has disappeared, both albumin and globulin gradually recover their normal serological behavior. These changes were not observed in patients with acute hemorrhagic nephritis. While globulin isolated from nephrotic serum was capable of stimulating antibody formation readily, the antiserum formed was not identical with that formed against globulin obtained from normal serum. These phenomena have been attributed to the presence of altered proteins in both albumin and globulin fractions of the serum.⁴³ Minimal amounts of altered albumin have been found in the serum in acute glomerulonephritis⁴³ while the urine of patients with rapidly progressing renal insufficiency in glomerulonephritis contains a high concentration of gamma-globulin (electrophoresis).⁹

It has been reported that the albumin fraction of the serum of nephrotic patients is not homogeneous, as is normal serum albumin, but contains molecules of varying size.¹³ Since the subfractions of lower molecular size pass more abundantly into the urine, the mean molecular weight of the urine albumin may be only one-half that of the serum albumin and that of the urine globulin only one-third that of the serum globulins.¹³

Fischer believed that urinary proteins are derived exclusively from the renal cells. Whereas it seems likely that protein may appear in the urine as a result of extensive degeneration of renal epithelium, it is quite evident that the quantity so derived must be extremely small as compared with that which comes from the blood plasma. Furthermore, in spite of the fact that the most marked degrees of proteinuria are found in those disorders in which the demonstrable pathologic lesions are predominantly those of degeneration of the tubular epithelium, the weight of experimental evidence supports the view that by far the greatest part of the urinary protein is derived by passage of plasma proteins through the glomerular membrane.

Since the urine normally contains minute quantities of albumin, the term "albuminuria" implies rather a quantitative

than a qualitative deviation from the normal. This term is applied to the presence in the urine of abnormally large quantities of albumin, detectable by the commonly employed qualitative tests.

Functional Albuminuria. Under this heading are placed cases of albuminuria apparently not dependent upon organic disease. The urine is otherwise normal, renal functional activity is unimpaired, the degree of albuminuria is usually slight (below 500 mg. per 100 cc.), the condition is usually transitory and occurs most commonly in young individuals, whose subsequent medical history is negative.

(1) *Following Severe Exercise.* Temporary albuminuria of varying degree may occur following strenuous muscular exercise in individuals unaccustomed to such activity. It has been observed in raw recruits after forced marches, in football players, bicycle racers, Marathon runners and other athletes if the degree of exertion exceeds that to which the individual is accustomed. This type of functional albuminuria may be associated with the appearance of casts and even red blood cells in the urine. It is questionable whether the term "functional" is applicable to such cases as the presence of temporary renal damage cannot be ruled out. However, these individuals are otherwise normal in every respect and the condition is transitory.

(2) *Severe Mental Strain.*

(3) *Prolonged Exposure to Cold* (cold baths).

(4) *Alimentary Albuminuria.* The ingestion of excessive quantities of native protein, particularly egg albumin, may be followed, in two to three hours, by the appearance of albumin in the urine.

(5) *Essential Albuminuria.* Posner proposed the term "essential albuminuria" to include those cases variously designated as albuminuria of adolescence, cyclic albuminuria, postural or orthostatic albuminuria and intermittent albuminuria. This type of albuminuria is of great clinical importance because of its comparative frequency and because of the fact that it is commonly erroneously considered to be an evidence of renal disease. It is usually first discovered in the course of routine examination of applicants for insurance.

The condition occurs most frequently between the ages of fourteen and eighteen, more commonly in boys than in girls, particularly in those presenting manifestations of autonomic imbalance and vasomotor instability. It may be present in several members of the same family. The blood pressure, particularly the diastolic pressure, is frequently low. Lordosis is a prominent feature in certain cases. The term "orthostatic"

is applied to those cases in which albuminuria is present during periods of ordinary activity in the erect posture and disappears upon the assumption of the recumbent posture. Heredity, lordosis, abnormal renal circulation, movable kidney and vasomotor instability appear to be important etiologic factors in many of these cases. In certain instances the condition persists into adult life, in others it disappears spontaneously.

As in most of the previously mentioned types of "functional" albuminuria, the essential etiologic factor is probably some disturbance of renal circulation, either in the large vessels (movable kidney, lordosis) or in the renal arterioles (vasomotor instability). The occurrence of temporary albuminuria following experimentally produced circulatory changes in the renal vessels has been frequently demonstrated. The clinical importance of this type of albuminuria lies in the fact that it is not associated with nor followed by renal disease. So far as is known, these individuals are not predisposed to the development of nephritis or nephrosis and the expectation of life is not affected by the presence of essential albuminuria.

(6) *Premenstrual Albuminuria*

(7) *Albuminuria of Pregnancy.* Albuminuria may occur in 30 to 50 per cent of women during uncomplicated pregnancy and labor, disappearing immediately after parturition.

Organic Albuminuria. Albuminuria may result from (a) qualitative alteration in the plasma albumin (p. 89), usually but not necessarily in association with a glomerular lesion, (b) renal changes secondary to abnormalities in organs other than the urinary tract (prerenal albuminuria), (c) primary renal disease (renal albuminuria) and (d) disease of the lower urinary passages and contiguous structures (postrenal albuminuria).

The organic causes of albuminuria may be conveniently discussed under three headings: (a) Prerenal, (b) postrenal and (c) renal.

(a) *Prerenal Albuminuria.* The term "prerenal" cannot be applied in a strict sense, for most of the prerenal factors which cause albuminuria do so by producing changes in the kidneys. However, from the standpoint of the primary condition the use of this term is justifiable and appears in many respects advantageous.

(1) **CARDIAC DISEASE** In the decompensated stages of myocardial disease, with passive congestion of the kidneys, albuminuria of varying degree develops. The quantity of albumin eliminated is directly dependent upon the degree of renal circulatory embarrassment, and, unless the state of passive congestion is unduly prolonged, with consequent organic change

in the kidneys, the albuminuria disappears upon reestablishment of circulatory efficiency.

(2) ASCITES due to local intra-abdominal disease, unassociated with nephritis or myocardial failure, may cause albuminuria, presumably by pressure upon the renal veins, causing renal congestion.

(3) INTRA-ABDOMINAL TUMORS may produce albuminuria if they are so situated as to cause pressure upon the renal veins.

(4) FEBRILE ALBUMINURIA. Fever, regardless of the cause, may be associated with slight albuminuria. This is due to the production of slight glomerular and tubular changes which are not permanent, being essentially nephrotic rather than nephritic in nature (cloudy swelling) and rapidly subsiding following the restoration of normal body temperature. Febrile albuminuria is commonly observed in pneumonia, typhoid fever, rheumatic fever, malaria and the acute infectious diseases of childhood. In the latter, particularly in scarlet fever, this condition must be carefully differentiated from a complicating nephritis.

(5) CONVULSIVE DISORDERS. Albumin may appear in the urine during and after convulsions from any cause, including brain tumor, tetanus, epilepsy and meningitis. Albuminuria may also occur in coma due to cerebral vascular accidents, particularly cerebral or meningeal hemorrhage, in which conditions large quantities of albumin may be eliminated (1-2 Gm. per 100 cc.).

(6) DISEASES OF BLOOD. Albuminuria of slight or moderate degree may occur in association with profound anemia, leukemia and purpura hemorrhagica. It is perhaps due to nutritional changes in the kidneys (nephrosis).

(7) HYPERTHYROIDISM. Albuminuria in hyperthyroidism is probably due to nephrosis or to vasomotor instability as in the case of certain types of functional albuminuria.

(8) INTESTINAL OBSTRUCTION. Albuminuria is present in most cases of acute intestinal obstruction (see Intestinal Obstruction, p. 235). It may be dependent upon renal changes resulting from the presence in the blood stream of toxic protein products or from the state of alkalosis which is a prominent feature of the condition.

(9) HEPATIC DISEASE AND JAUNDICE. Jaundice is not infrequently accompanied by the elimination of small quantities of albumin in the urine. This has been attributed to a toxic or irritant effect of the circulating bile pigments or bile acids upon the renal glomerular membrane or the tubular epithelium. In addition to this factor, it may be that the existing state of hepatic dysfunction or functional insufficiency results in some alteration in the nature or mode of combination of the plasma

proteins which causes them to be eliminated by the kidneys. This problem was referred to in discussing the nature and origin of urinary proteins.

(10) DRUGS which cause renal irritation may give rise to albuminuria. Among these are turpentine, cantharides, phosphorus, arsenic, mercury, quinine, copaiba, salicylic acid, phenol, bismuth, lead, ether, chloroform, chromates, oxalates, zinc, opiates, apiol, sandalwood, oil, lysol, naphthols, radium, squill barbiturates and sulfonamides. In the majority of instances the renal lesion is largely degenerative in nature (nephrosis).

(11) LIPOID NEPHROSIS. Because of the existing discordant views regarding the essential nature of this disorder, it will be considered in the group of albuminurias of renal origin, although it is possible that it may belong in the prerenal group.

(b) *Postrenal Albuminuria*. Under this designation may be placed the causes of so-called "false" albuminuria, a term frequently applied to the addition of albumin to the urine at some point beyond the uriniferous tubule. This group includes, therefore, inflammatory and degenerative lesions of the renal pelvis, ureter, bladder, prostate and urethra. The possibility of contamination of the urine by vaginal secretions and discharges must be carefully excluded in considering the cause of albuminuria in women. The commonly employed tests for albumin will, of course, yield positive results in the presence of blood, the source of which frequently resides in the lower urinary tract. Inflammatory exudates are rich in protein and are therefore often responsible for albuminuria of this type. Consequently, urine containing pus will practically always contain albumin. Goldberg states that ordinarily, for each 100,000 leukocytes per 2 cc. of urine, 0.1 per cent of albumin may be expected. This observation is at times of importance in determining the presence or absence of albumin of renal origin in urine containing pus resulting from a lower urinary inflammatory process.

(c) *Renal Albuminuria*. (1) DESTRUCTIVE LESIONS OF KIDNEY. Albuminuria may occur in tuberculosis, carcinoma, pyelonephritis and polycystic disease of the kidney and in hypernephroma and other lesions which are characterized by destruction or invasion of kidney tissue. In many cases, however, the urinary findings are normal. Renal infarction is usually associated with albuminuria which is at first marked but rapidly subsides.

(2) GLOMERULONEPHRITIS AND NEPHROSCLEROSIS. Albuminuria is an almost constant feature of acute glomerulonephritis. It may be of moderate or severe degree; the amount of albumin does not necessarily parallel the severity of the condition.

Albuminuria is one of the most persistent manifestations of acute nephritis, being usually present after other manifestations (except microscopic hematuria) have disappeared. In the latent stages of glomerulonephritis albuminuria may be the only indication of the renal lesion. In the early stages of chronic glomerulonephritis large quantities of albumin may be eliminated over long periods of time. With the development of impairment of renal function the albuminuria diminishes and in the later stages of the disease may be extremely slight. This change is due to the progressive impairment of the ability of the kidney to eliminate not only the substances normally present in the urine but also plasma albumin and other proteins.

In nephrosclerosis (essential hypertension) albuminuria varies both in incidence and in degree. In many patients with essential hypertension albuminuria is absent except during periods of myocardial failure with renal congestion. It may be intermittent and slight in amount. In so-called "malignant" hypertension, with rapid progression of the vascular changes in the kidney and with consequent nutritional changes in the glomeruli, relatively large quantities of albumin may be eliminated. With increasing renal functional impairment, however, albuminuria may diminish as in the case of chronic glomerulonephritis.

(3) NEPHROSIS (see p. 400). The term "nephrosis" has been applied to diseases characterized anatomically by primarily degenerative lesions of the renal parenchyma. The nephroses are subdivided into four groups by Fishberg: (a) larval nephroses, including those forms due to fevers, diabetes, jaundice, hyperthyroidism, pernicious anemia and various drugs; (b) necrotizing nephroses, including those forms due to chemical agents, chiefly mercury, bismuth, and, less frequently, arsenic and the barbituric acid series of hypnotics, and those due, in rare instances, to severe acute infections (cholera, typhoid fever, diphtheria); (c) chronic nephrosis, the most characteristic form being so-called "lipoid" nephrosis; (d) amyloid disease of the kidneys.

Albuminuria is a constant feature of nephrosis, except in those acute necrotizing forms which are frequently accompanied by anuria. If any urine is eliminated, it will practically always be found to contain albumin. The quantity of albumin varies. In the mild forms of larval nephrosis and in certain cases of necrotizing nephrosis albuminuria may be slight; in other cases, however, it may be marked. Large amounts of albumin are usually excreted in lipoid nephrosis and in amyloid disease of the kidneys. Only in rare instances are small quantities eliminated. As has been mentioned previously, the urinary protein

in these conditions consists largely of albumin, the albumin: globulin ratio being high (above 10). If an existing nephrosis, with purely or predominantly tubular lesions, becomes complicated by a superimposed nephritis with glomerular lesions, the albuminuria may diminish, and, with progressive renal functional impairment, only small quantities of albumin may be eliminated, the urinary picture being similar to that observed in chronic glomerulonephritis.

(4) ECLAMPSIA GRAVIDARUM. Large amounts of albumin may be eliminated in true eclampsia, unassociated with glomerulonephritis or nephrosis. This is probably dependent upon renal vascular damage or, perhaps, may result from the hepatic lesions which constitute an important pathologic feature of that condition.

Quantity of Protein in Urine

As has been indicated, the amount of protein eliminated in the urine is exceedingly variable. Addis believes that the upper limit of normal proteinuria is about 30 mg. for the twelve-hour-day period. Others state that as much as 75 mg. may be eliminated in twenty-four hours by normal individuals under normal conditions. In benign, functional or physiologic albuminuria, the mixed twenty-four-hour-specimen seldom contains more than 0.2 per cent of protein, although in some cases as much as 0.5 per cent has been observed. Immediately after severe exercise, however, and in orthostatic albuminuria shortly after assuming the erect posture, specimens of urine may contain 1 per cent or more of protein.

The largest amounts of protein are usually present in the urine of patients with chronic nephrosis and amyloid nephrosis, in which conditions 2-5 per cent or more of protein may be eliminated. More than 110 Gm have been eliminated in twenty-four hours, the usual quantity varying from 5-60 Gm. with an average daily loss of 15-20 Gm. Some investigators have observed an increase in urinary protein in these cases during periods of excessive protein intake. This is particularly true in those instances in which the daily output is greater than 20 Gm. In most cases of nephrosis, however, large amounts of protein may be ingested without an associated increase in albuminuria; in certain instances of lipoid nephrosis albuminuria may diminish following the administration of a high protein diet with consequent increase in the concentration of albumin in the blood plasma.

In acute glomerulonephritis the urinary protein usually varies from 0.2-1.0 per cent, but may exceed 2 per cent in rare

instances. In chronic glomerulonephritis and in essential hypertension the figures usually vary from 0.2-0.5 per cent; in some cases, particularly in those instances of nephritis with a nephrotic component, the urinary protein may be greatly increased and approach the amounts observed in pure nephrosis. With the supervention of renal insufficiency, however, the quantity of protein in the urine in all types of nephritis is usually diminished. According to Keutmann, increases in proteinuria in Bright's disease may be explained by the presence of one or more of the following factors: (a) increase in glomerular permeability; (b) increase in the rate of glomerular filtration; (c) the presence in the diet or the body reserves of more new material from which plasma proteins may be constructed; (d) artificial increase in the concentration of plasma protein, such as follows transfusion. It would appear that, in addition to increased severity of damage to the glomerular capillaries, an increase in the amount of protein in the urine may be effected in some instances by an increase in protein intake and even by actual improvement in renal function, as indicated by the urea clearance test. Such findings emphasize the fact stated above, that the quantity of albumin in the urine does not parallel the severity of renal damage.

In cases in which the urine contains large quantities of protein, this substance may have a significant effect upon the urinary specific gravity. It has been found that 1 Gm. of albumin per 100 cc. increases the specific gravity by 0.003. Under such circumstances, correction must be made for the amount of albumin in the urine when performing urine concentration tests, which depend upon accurate estimation of urinary specific gravity. If such correction is not made, results will be obtained which may be misleading.

Other Proteins in Urine

Nucleoprotein in Urine. Nucleoprotein is present in the urine in abnormal amounts in inflammatory conditions of the lower urinary passages, particularly cystitis and pyelitis; it may also be found in nephritis. The term nucleoprotein is incorrectly applied to include other protein substances such as mucin and phosphoprotein which may appear in the urine in increased amounts.

Proteoses and Peptones in Urine. Proteoses or some closely related substances are frequently found in the urine in certain febrile disorders, particularly pneumonia, diphtheria and pulmonary tuberculosis, as well as in peptic ulcer, carcinoma and

osteomalacia. Peptonuria is extremely rare, most of the reported cases representing, perhaps, proteosuria.

Bence-Jones Proteinuria. At times proteins are found in the urine which do not give the precipitin reactions for any of the proteins found in normal blood. One of the most significant of these is the Bence-Jones protein which occurs in the urine in some cases of multiple myeloma, myelogenic osteosarcoma, carcinomatous metastases to the bone marrow and, less frequently, in leukemia. This substance differs from all other proteins occurring in urine in that it precipitates at a relatively low temperature ($50^{\circ}\text{C}.$ – $60^{\circ}\text{C}.$) and is partially or completely dissolved at $100^{\circ}\text{C}.$, the precipitate reappearing upon cooling. The condition has therefore been termed "thermolytic albuminuria." It is believed by many that Bence-Jones protein is formed in the bone marrow. Bence-Jones protein appears in the urine in 60 to 70 per cent of patients with multiple myeloma. Protein in the urine in such cases may consist entirely of Bence-Jones protein, entirely of serum protein, or of a mixture of the two proteins. As stated by Bell, Bence-Jones protein is found more frequently in advanced than in early stages of the disease and, when present, it usually constitutes the greater part or all of the urinary protein. A few cases of multiple myeloma have been reported in which the urine contained no protein. Bence-Jones protein appears to be globulin in nature. Its molecular weight appears to be relatively low (about 30,000) as compared with other globulins found normally in the serum. The occasional failure to detect this protein in the urine may be due in part to the fact that the typical precipitation reactions mentioned above are markedly influenced by the acidity and the salt concentration of the urine, which factors may not always be favorable for the appearance of these reactions.

Hemoglobinuria. (p. 97.) Hemoglobinuria is the term applied to the presence of free hemoglobin in the urine, in contradistinction to hematuria which refers to the presence of red blood corpuscles in the urine. The excretion of hemoglobin by the kidneys is almost invariably preceded by the occurrence of hemoglobinemia, an excessive amount of free hemoglobin in the circulating blood plasma. Hemoglobinuria is therefore dependent upon excessive hemolysis. It has been estimated that it occurs only after the plasma contains more than 60–150 mg. of free hemoglobin per kilogram of body weight. The clinical conditions in which this occurs are presented elsewhere (Hemoglobinemia, p. 97).

Before making a diagnosis of hemoglobinuria it is, of course, essential to exclude the possibility that the condition may in

reality be hematuria, the red corpuscles having been partly or completely hemolyzed in the bladder or after elimination of the urine. This is particularly apt to occur in alkaline or ammoniacal urine.

Urinary Nonprotein Nitrogen

Investigation of the nonprotein nitrogenous constituents of the urine in disease has been largely superseded by the quantitative determination of these substances in the blood. However, particularly in nephritis, the study of both blood and urinary nonprotein nitrogenous elements may yield valuable information. Since the amount of urinary nitrogen depends primarily upon the quantity of protein ingested, it is obvious that no clinical significance can be attached to the nitrogenous output unless the intake be determined simultaneously. Even under such circumstances accurate information cannot be obtained in many instances because of the fact that nitrogen equilibrium may be established at various levels of blood nonprotein nitrogen. Before drawing conclusions from alterations in urinary nitrogen, therefore, several factors must be carefully investigated.

Urinary Urea. Urea administered to normal individuals is promptly eliminated because the storage capacity of the body for nitrogen is limited. In certain patients with nephritis the elimination of excessive amounts of ingested urea may be delayed for varying periods of time. In some cases, however, no deviation from the normal can be demonstrated. In patients with chronic nephritis in whom retention of nitrogen occurs following the ingestion of excessive quantities of protein, the retained nitrogen frequently cannot be entirely accounted for by the concentration of nonprotein nitrogen in the blood, which may or may not be increased; it is believed that in such cases the excess nitrogen is retained in the tissues, chiefly in the liver and muscles.

In many cases of nephritis the ability of the kidneys to concentrate urea is impaired although the amount eliminated in twenty-four hours may be normal. The normal individual is able to concentrate urea to the extent of 2 per cent or more in the second hour after the ingestion of 15 Gm. of urea dissolved in 100 cc. of water. If renal function is impaired the urea concentration in the urine is below this figure (see p. 361).

Perhaps the most important clinical applications of studies of urea elimination have resulted from the investigations of Addis and of Austin, Stillman, Van Slyke, Möller, McIntosh and MacKay. As a result of these studies it has been shown that with urine volumes below about 2 cc. per minute (the augmentation

limit) the blood urea clearance increases in direct proportion to the square root of the urine volume. With urinary outputs below this figure the volume of blood which is cleared of urea in one minute was found to vary, in normal individuals, from 41 to 65 cc., with a mean of 54 cc. (standard clearance). When the rate of urine excretion exceeds 2 cc. per minute, the urea clearance, having attained its maximum at that point, is unaffected by further increase in urine volume. Under such circumstances, the volume of blood which is cleared of urea in one minute was found to vary from 64 to 99 cc. with a mean of about 75 cc. (maximum clearance) (see p. 372). Van Slyke and his associates found that a decrease in the urea clearance could be demonstrated in patients with renal disease before evidence of renal functional impairment could be obtained by any other method. This subject will be considered in greater detail in the discussion of tests of renal function (p. 375).

In hepatic insufficiency, owing to decreased formation of urea from amino acids, the proportion of urinary nitrogen occurring as urea may be decreased. Urea normally constitutes about 60 per cent of the total urinary nitrogen on a low protein diet and 90 per cent on a high protein diet, the average figure being about 80 per cent. Figures of 50 per cent may be obtained in acute hepatic disease even with an adequate protein intake. In many cases, however, the urinary nitrogen partition is normal.

A relative decrease in the urinary urea fraction may also occur in conditions associated with severe acidosis. Under such circumstances the low proportion of urea is due to the great increase in urinary ammonia, formed by the kidney, probably from amino acids, in an attempt to combat the acidosis by conserving the alkali reserve (p. 275). Gaebler has found that the injection of anterior pituitary extract into adult dogs maintained on a high protein diet is followed, within twenty-four hours, by a marked decrease in urinary nitrogen, which persists for several days; this was due practically entirely to a diminution in urea excretion. This finding is of particular interest in view of its possible relation to the action of the growth hormone of the anterior hypophysis (p. 85).

An increase (above nitrogen intake) in the amount of urea eliminated in the urine may occur in disorders associated with excessive tissue catabolism, as in prolonged wasting diseases and febrile states, urea (or urea plus ammonia) comprising by far the largest portion of the increased nitrogen output. The outstanding exception is severe hepatic necrosis, in which the deamination mechanism in the liver is impaired, resulting in

excretion of relatively large amounts of amino acids and correspondingly smaller amounts of urea (as low as 10-50 per cent of the total urine *N*).

Uric Acid in Urine. The determination of the uric acid content of urine is of little practical significance. The factors which influence its variation under normal conditions have been considered (p. 82). Uric acid excretion diminishes several days before an acute attack of gout, with a sharp increase beginning one to two days prior to the attack, persisting throughout it and gradually returning to normal during the period of recovery.

The administration of atophan (cinchophen) is followed characteristically by an increased elimination of uric acid (p. 83). Uric acid is also frequently increased in amount in leukemia, particularly chronic myelogenous leukemia, because of excessive nuclear catabolism. It may be similarly increased during the early periods of remission in pernicious anemia, especially following the administration of liver or liver extract, and at times in eclampsia. Uric acid calculi may develop in highly acid urine with a high uric acid content because of its relative insolubility in acid solutions.

Creatine and Creatinine in Urine. The excretion of creatine in the urine under normal conditions has been discussed elsewhere (p. 83). The investigation of this factor has acquired significance particularly in the study of metabolic changes in certain myopathies. The early work of Fiske and Subbarow and the Eggletons established the important relation of creatine metabolism to muscular function. Fiske and Subbarow found an unstable compound containing phosphoric acid, and creatine in fresh, excised rat muscle, which soon began to disappear and, in a few hours after removal of the muscle, was no longer demonstrable; as it disappeared, free phosphoric acid and creatine appeared in molecular ratio to one another. This unstable compound of phosphoric acid and creatine was diminished by stimulation. Stimulation during arrest of the circulation through the muscle caused the compound to break up completely and, when the blood supply was restored and the muscle allowed to rest, the combination of phosphoric acid with creatinine was once more renewed, the quantity of the compound increasing steadily during the period of rest. Observations such as these indicate that creatine has an important bearing on the mechanism of muscular contraction.

Excessive creatinuria may occur in starvation, febrile and wasting diseases, diabetes mellitus, in eunuchoids and castrates, in postencephalitic and Parkinsonian rigidity¹¹⁴ and rather consistently, but not invariably, in certain myopathies (myotonia

congenita, myasthenia gravis, various forms of myositis, congenital muscle dystrophies, congenital muscular hypertrophy and secondary muscle atrophy). Testosterone tends to diminish creatinuria in eunuchoids and castrates. Methyltestosterone increases it.

Of particular interest in this connection is the influence of glycine upon the clinical and metabolic course of certain of these myopathies, particularly myasthenia gravis. The administration of large doses of glycine results, in certain of these cases, in a 100-1000 per cent increase in creatine excretion. A similar increase in creatinuria also occurs in the majority of normal subjects after the ingestion of glycine. After a period of several weeks the creatinuria begins to decrease in most instances, eventually falling to almost control levels despite the continued administration of glycine. The observation has been made that cases of primary myopathy in which the average creatinuria increased more than 50 per cent above the control level after the administration of glycine showed both subjective and objective improvement, provided this increased creatinuria disappeared within a few weeks. Little or no improvement was observed in cases in which the increase in creatinuria was less than 50 per cent.

Excessive creatinuria has also been observed in hyperthyroidism, following the administration of thyroid extract, during the menstrual period and in pregnancy, and when carbohydrate is excluded from the diet. It has also been observed after fractures (to 500 mg. daily). It disappears gradually during the period of healing of the fracture and after the resumption of activity.⁵²

As has been stated (p. 83), the output of creatinine is remarkably constant under normal conditions and is an index of endogenous protein metabolism. As such it is essentially independent of diet and muscular activity. The elimination of creatinine is increased in conditions associated with increased tissue catabolism, as in fevers, and is decreased in the presence of marked muscular atrophy.

It has been found that in the primary myopathies the diminished creatinuria which occurs as a secondary phenomenon following the administration of glycine is accompanied by an increased excretion of creatinine and an improvement in the patient's ability to retain ingested creatine.

Preformed creatinine, administered to normal individuals, is promptly eliminated. Major found that if 500 mg. of creatinine, in buffered solution, are injected intravenously, 15-18 mg. are eliminated in the urine within fifteen minutes. In the presence

of chronic nephritis the excess creatinine is eliminated very slowly.

Creatine Tolerance. When 1.32 or 2.64 Gm. of creatine hydrate (equivalent to 1 and 2 Gm., respectively, of creatine) are ingested by a normal adult, about 80 per cent is retained by normal men and about 70 per cent by normal, nonpregnant women; about 20 and 30 per cent, respectively, are excreted in the urine during the subsequent twenty-four hours. Diminished tolerance (*i.e.*, excessive excretion) is present in conditions accompanied by spontaneous creatinuria, mentioned above, and in mild cases of these disorders in the absence of creatinuria under ordinary dietary conditions.

Creatine tolerance is decreased in the majority of patients with hyperthyroidism and tends to be increased (excessive retention of administered creatine) in hypothyroidism.^{111,122} This procedure may be of value in the diagnosis of hyperthyroidism or hypothyroidism in children, in whom it may be difficult to secure accurate determinations of basal metabolic rate. In children, correction must be made for normal creatinuria, which averages 4.2 mg. daily per kilogram of body weight on a meat-free diet, providing adequate calories and 2 Gm. of protein per kilogram.¹²³

Amino Acids in Urine. An increase in the amino acid content of the urine may occur in conditions associated with an increase in the amino acid content of the blood. This occurs chiefly in those conditions in which hepatic function is impaired and in those characterized by extensive tissue autolysis. High figures are therefore obtained in acute toxic necrosis of the liver as present in acute yellow atrophy of the liver, eclampsia, and in chloroform, phosphorus, cinchophen, arsenic and carbon tetrachloride poisoning. Under such circumstances leucine, tyrosine, glycine, arginine, phenylalanine and other amino acids may be present in the urine in large quantities. This increased elimination is due partly to decreased deamination of amino acids in the liver and partly to extensive autolysis of proteins of the liver itself with consequent formation of amino acids in large amounts. Urinary amino acids are also increased in conditions associated with excessive tissue wasting as in protracted fevers (typhoid)

Specific amino acids, or products of amino acid metabolism, may appear in the urine in certain conditions.

(a) *Tyrosinuria and Phenylketonuria.* Transitory, minimal or moderate tyrosinuria may occur in subacute atrophy of the liver and toxic hepatitis, and, rarely, in prolonged calculous obstructive jaundice. Continuous, massive tyrosinuria (0.9-2

Gm. daily) occurs in some but not all cases of severe, fatal, acute hepatic necrosis, and is of serious prognostic significance.⁷² It may occur also in the presence of extrahepatic foci of autolysis, as in degenerating lung tumors or extensive sloughing of the skin. The source of the tyrosine is believed to be largely the degenerating tissue protein, but in the case of severe hepatic functional impairment, diminished deamination may contribute to the increased tyrosinuria.

Tyrosinuria may occur also as an "inborn error of metabolism" (Garrod), tyrosine being accompanied by other amino acids, such as dihydroxyphenylalanine, hydroxyphenylpyruvic acid and hydroxyphenyl acetic acid.⁷⁹

Phenylpyruvic acid has been found in the urine of certain patients with mental deficiency and manifestations of extrapyramidal system disturbance.^{34,56} Urine containing this substance gives a dark green color with ferric chloride solution. The condition is apparently due to impairment of the metabolism of phenylalanine and is probably transmitted as a recessive Mendelian characteristic.

P-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids have been found in the urine of premature infants fed diets of vitamin C-free cow's milk containing 5 Gm. or more of protein per kilogram. These substances disappeared after administration of vitamin C.⁷¹

(b) *Cystinuria*. Small amounts of cystine (0.8-84 mg. daily) may appear in normal urine.⁸⁰ The term cystinuria is applied to one of the "inborn errors of metabolism"³⁹ in which this substance is present consistently in the urine in abnormally large amounts (0.4-1 Gm. daily). The neutral (protein) sulfur of the urine is increased and inorganic sulfur is usually decreased. In such individuals, the urine cystine is increased by administration of protein, methionine or cysteine, but not cystine, indicating that the difficulty does not lie in the breakdown of the latter but rather in the handling of the former amino acids.¹⁴ The condition is not necessarily of clinical importance unless precipitation of crystalline cystine, which is particularly insoluble at the normal urine pH, results in the formation of cystine calculi in the urinary tract. It shows a familial and congenital tendency and is inherited as a recessive Mendelian characteristic.

(c) *Alkaptonuria*. This condition is one of the rare "inborn errors of metabolism,"³⁹ with a familial and congenital tendency, inherited as a Mendelian recessive characteristic. It is characterized by the urinary excretion of homogentistic acid, due to an abnormality of the intermediary metabolism of phenyl-

alanine and tyrosine. Homogentisic acid has been found in the urine of normal subjects given tyrosine and a diet deficient in vitamin C, disappearing after administration of the vitamin. The latter has no effect upon clinical alkaptonuria.^{101,102} Excretion of homogentisic acid in such subjects is increased by administration of phenylalanine or tyrosine or their corresponding keto acids and by ingestion of proteins containing a high percentage of these amino acids. This condition is occasionally accompanied by ochronosis.

(d) *Melanuria*. Melanin is a dark, brown-black, sulfur-containing pigment, normally found in the hair, skin, ciliary body, choroid of the eye, pigment layer of the retina and various nerve cells. Its exact chemical structure is not known, but it apparently is derived from tyrosine which, through the action of tyrosinase, forms dihydroxyphenylalanine (dopa), the latter, a colorless substance; giving rise to a series of oxidation products terminating in melanin.^{11,12b}

Melanuria occurs almost exclusively in the presence of melanotic sarcoma (in about 20 per cent of cases), usually when extensive metastases are present. It may be regarded as presumptive evidence of this condition although it has been reported, in rare instances, in terminal stages of hepatic cirrhosis^{13a} and in intestinal obstruction, pernicious anemia, pneumonia, acute pancreatitis and diabetes mellitus.^{13b} It is occasionally accompanied by ochronosis.

Ammonia in Urine. It was formerly believed that urinary ammonia was a product chiefly of deamination of amino acids and represented that portion which had not undergone synthesis into urea in the liver. It is now known that such is not the case and that the ammonia present in the urine is formed largely in the kidneys, probably from amino acids, for purposes of neutralization of excreted acids. The quantity of ammonia in the urine consequently bears no relation to that in the blood. The work of several investigators, notably Nash and Benedict, has demonstrated that the neutralization of acids by ammonia is not a generalized tissue phenomenon, but is a function of the kidney alone (Lusk).

Urinary ammonia is therefore increased in many conditions associated with acidosis (diabetes, starvation, dehydration, vomiting, diarrhea, etc.), this increase constituting one of the important compensatory mechanisms of the body whereby acid bodies may be neutralized without the loss of excessive amounts of blood and tissue alkali. The acidosis of nephritis, in which the ammonia-forming function of the renal tubular epithelium is impaired, constitutes one exception to this general rule. Great

increases are characteristically noted in the acidosis of diabetes, of infants, and of phosphorus and arsenic poisoning, and in eclampsia and acute yellow atrophy of the liver. The ammonia content of the urine is decreased in alkalosis. An increase may result from bacterial decomposition of urea in subjects with bladder retention and urinary tract infection. (particularly cystitis).

Because of the rôle of urinary ammonia in the regulation of the acid-base equilibrium, the factor, ammonia plus titratable acid of the urine, may be utilized as an index of the state of this equilibrium, except in the presence of renal functional impairment or acidosis due to retention of carbonic or phosphoric acids. Normal and abnormal values, in terms of twenty-four-hour excretion of $N/10 \text{ HCl} + \text{NH}_4$ per kilogram of body weight are as follows:¹¹⁶ normal resting adult, 0-27 cc.; mild acidosis, 27-65 cc.; moderate to severe acidosis, 65-100 cc.; severe acidosis, over 100 cc. (p. 292).

TABLE 3
INFLUENCE OF HIGH AND LOW PROTEIN DIETS ON THE RELATIVE AMOUNTS OF THE NITROGENOUS CONSTITUENTS OF THE URINE

Food		Composition of the urine in grams					
In grams	In calories	Urea N	Ammonia N	Uric acid N	Creatinine N	Undetermined N	Total N
Protein, 118 = 19N Fat, 148 Carbohydrate, 225	2786	14.70 57.5 per cent	0.49 3.0 per cent	0.18 1.1 per cent	0.58 3.6 per cent	0.85 4.9 per cent	16.8
Protein, 6 = 1N Fat, 52 Carbohydrate, 400	2153	2.20 61.7 per cent	0.42 11.3 per cent	0.09 2.5 per cent	0.60 17.2 per cent	0.27 7.3 per cent	3.6

Lusk, G. Science of Nutrition, 4th ed., p. 251, W. B. Saunders Co., 1928. After Folin, O., Am. J. Physiol., 23, 117, 1905.

Urinary Nitrogen Partition. The preceding table taken from Lusk and based upon an experiment reported by Folin illustrates the relative amounts and proportions of the several nonprotein nitrogenous constituents of the urine excreted by normal individuals upon high and low protein diets.

As stated by Lusk, "A study of this table will reveal the fact that if a man ingest a diet containing a medium amount of protein, and again one that is nearly free from protein, the difference in the character of the urine in the two cases is almost exclusively due to a difference in the output of urea. The quantity of creatinine eliminated remains independent of the quan-

tity of protein metabolized, and the same thing holds true, as a rule, for uric acid." The determination of the urinary nitrogen partition is employed clinically particularly in the study of hepatic function. If hepatic function is impaired the proportion of urea *N* in the urine will be relatively low and that of amino acid *N* will be increased. In acidosis (except nephritis), urea *N* will be subnormal and ammonia and amino acid *N* increased. The study of the urinary nitrogen partition has been largely replaced by improved methods of study of renal and hepatic function and by more accurate methods of investigating alterations in the acid-base equilibrium. It must be remembered, for example, that an increase in urinary ammonia may occur in the alkalotic state associated with the anoxemia of high altitudes. The failure to recognize facts such as this frequently leads to misinterpretation of information supplied by limited investigative procedures.

Congo Red Test for Amyloidosis

Congo red, injected intravenously, is removed from the blood relatively slowly, about 70-90 per cent of the quantity injected remaining in the blood at the end of one hour in normal individuals. Bennhold found that in the presence of amyloid disease the dye disappears from the blood more rapidly than normally, less than 40 per cent of the quantity injected remaining in the blood stream at the end of one hour. Under normal conditions practically all of the dye is excreted by the liver in the bile. The increased rapidity of its disappearance in amyloid disease is generally attributed to adsorption of the dye by the amyloid material and to its increased filtration from the blood through the damaged capillary endothelium (Bennhold).

This procedure is widely employed for the diagnosis of amyloid disease and is an extremely valuable test if certain complicating factors are taken into consideration. It has been found that the persistence of Congo red in the blood stream is dependent to a certain extent upon a normal concentration of plasma protein, particularly albumin, to which the dye is adsorbed.²⁷ In the nephrotic syndrome, with marked albuminuria and a lowered plasma albumin concentration, the dye disappears from the blood with abnormal rapidity, as in the case of amyloid disease, a large proportion of it appearing in the urine, however, adsorbed to albumin. In the presence of marked albuminuria, therefore, the interpretation of subnormal retention of Congo red in the blood is difficult, but in amyloid disease much less of the dye appears in the urine as a rule than in the case of the nephrotic syndrome. Occasionally, findings suggestive of the presence of amyloid disease are obtained in

patients with low plasma albumin concentration in the absence of albuminuria. Recent studies indicate that the Congo red test may be interpreted as strong confirmatory evidence of the presence of amyloid disease when more than 90 per cent of the dye disappears from the blood within one hour, in the absence of the elimination of significant amounts in the urine. In exceptional cases of amyloid disease this test may yield essentially normal findings, and findings suggestive of amyloid disease may be obtained occasionally in cases in which no amyloid substance is present.^{72a} There is apparently no consistent relationship between the rapidity of removal of the dye from the blood and the extent of amyloid deposition in the tissues.

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Chapter III

Lipid Metabolism

THE term "lipid" is employed here, as by Bloor,⁸ to designate those substances which, in their general properties and particularly in their solubilities, resemble fats. The lipids are divided into three main classes: (1) true fats; (2) phospholipids, substances which yield, on hydrolysis, fatty acids or their derivatives, and which contain either nitrogen or nitrogen and phosphorus in their molecule; (3) sterols, the most important of which is cholesterol, which are unsaponifiable substances having no close chemical relation to fats.

FAT METABOLISM^{1,2,6,8,10}

Fats are triglycerides of fatty acids, consisting of the combination of three fatty acid radicles with one molecule of glycerol. Palmitic, stearic and oleic acids, the most important fatty acids in the animal organism, are straight-chain acids with an even number of carbon atoms, the first two being fully saturated and the last unsaturated.

Fat-splitting enzymes (lipases) are present in the secretions of the stomach, pancreas and small intestine. The most important of these is pancreatic lipase. Under conditions of normal gastric secretion, gastric lipase possesses little or no functional activity, but some degree of fat digestion may occur in the stomach if the acidity of the gastric juice is significantly decreased. Under the influence of these enzymes, fats are broken down into fatty acids and glycerol. Under normal conditions, the digestion of fat is considerably enhanced by the presence of bile salts in the intestine. These substances exert their influence in this connection in the following manner: (1) They serve as activators of pancreatic lipase. (2) They aid directly in the emulsification of ingested fat. Due to their property of lowering surface tension, in the presence of bile salts fat globules are reduced in size, and the total surface area exposed to the lipolytic enzyme is thereby increased, digestion being correspondingly facilitated. (3) They aid in dissolving fatty acids which, in solution, also lower the surface tension and favor finer emulsification. In combination with alkali, fatty acids form soaps which also aid in the emulsification process.

Fat is absorbed largely if not entirely as fatty acids and glycerol, the process of absorption beginning in the duodenum, proceeding more rapidly in the jejunum and being practically complete in the terminal ileum. Although it is possible that absorption may occur directly into the portal circulation, about 60 per cent of the absorbed fat can be recovered from the thoracic duct, the lymphatic channels certainly constituting the chief route of absorption of fat under normal conditions. Bile plays an important rôle in the absorption as in the digestion of fat. The bile salts form soluble addition compounds with fat or fat-soluble substances and it is now believed that one of the most important functions of bile is to facilitate the absorption of fatty substances by the formation of these bile acid addition compounds. During the process of absorption the fatty acids are resynthesized to neutral fat in the epithelial cells of the intestinal mucosa, the character of the newly formed fat being usually somewhat modified so as to more closely resemble the body fat. This modifying process is far from perfect, as is indicated by the marked variation in the character of the fat mixture in the tissues. This is in marked contrast to the uniform character of tissue proteins and the stores of carbohydrate represented by glycogen.

It now seems quite certain that the formation of phospholipids from absorbed fatty acids in the epithelial cells of the intestinal mucosa is one of the first important steps in the resynthesis of neutral fat. It appears probable, therefore, that the process of phosphorylation is important in the absorption of fat as it is in the absorption of glucose (p. 1). It has been shown that factors that interfere with phosphorylation also inhibit the formation of neutral fat.⁴⁹ Among these are adrenalectomy and the administration of phlorhizin or iodoacetic acid. As stated by Verzar, fat absorption is probably dependent upon an active process in the intestinal mucosa which synthesizes fatty acids into phospholipids and neutral fat and which is under the influence of the adrenal cortex. Available evidence suggests that the fatty acids resulting from the digestion of ingested fats displace the fatty acids of phospholipids in the intestinal epithelium and pass through the intestinal wall in this form, which is much more soluble in the body fluids than are the fatty acids as such. One of the chief functions of phospholipids in this connection, therefore, is in the transportation of fat across the intestinal membrane.

As stated above, the absorbed fat enters the blood chiefly by way of the thoracic duct. During the period of fat absorption there is an increase in the quantity of neutral fat and, at times,

of phospholipid in the blood stream. The increase in the latter is helieved to indicate that phospholipids serve as a means of transportation of fatty acids to the tissues. As stated by Sinclair, it appears likely that the immediate destination of the absorbed neutral fat is the fat depots in the tissues, in which it is stored until it is called upon to supply the necessary fuel for the body.

The important part played by the liver in the intermediary metabolism of fat is indicated by the following facts:

(1) Phospholipids and fats deposited in the liver contain fatty acids which are more unsaturated than are fatty acids in other tissues. The fact is now definitely established that fatty acids undergo desaturation in the organism, this phenomenon occurring probably to a large extent in the liver, although direct proof of this is not available.³⁰

(2) In many conditions, neutral fat or cholesterol esters may accumulate in the liver to a much greater extent than in other tissues. This may occur during pregnancy toxemias, pernicious anemia, diabetes mellitus, certain infectious processes, following the injection of pituitary extracts and the administration of certain poisons (phosphorus, carbon tetrachloride, chloroform, benzol), and under certain dietary conditions (forced fat feeding, cholesterol feeding, low intake of choline, betaine or other lipotropic factors).

(3) The liver is the chief if not the only site of formation of ketone bodies.^{28,29} This is indicated by the following observations: (a) Ketosis of depancreatized dogs diminishes sharply after removal of the liver. (b) The ketonemia which follows the injection of certain anterior pituitary extracts in intact animals does not occur after hepatectomy. The injection of the ketogenic hormone of the anterior hypophysis is followed by an extra accumulation of fat in the liver. This suggests that the extra fat in this organ is in process of conversion to ketone bodies.

In the process of combustion of fats in the body, fatty acids are broken down to carbon dioxide and water. This occurs chiefly although not entirely through the process of beta-oxidation. In other words, the oxidation of the fatty acids occurs at the beta-atom of the carbon chain, leaving it shorter by two carbon atoms. Oxidation of the molecule proceeds by the successive removal of two carbon atoms. The concentration of fat and fatty acids in the blood under normal and abnormal conditions will be considered subsequently (pp. 143 ff.).

Fats are excreted in relatively large amounts by the intestinal mucosa, to a less extent by the bile, and in minute amounts by the skin.

FAT IN FECES

Fat is normally present in the feces in three forms, soap-fat (combined fatty acids), free fatty acids and neutral fat, the relative proportion of each being dependent somewhat upon the efficiency of fat digestion and absorption. There are two methods commonly employed for the quantitative analysis of fecal fat, the dry method of Cammidge and the wet methods of Saxon and of Fowweather. The most popular clinical method is that advocated by Cammidge, which has been attacked as unsound by Fowweather who bases his opinion upon the following observations: "Any method in which the stools are dried by heat before analysis is open to serious objections. Stools are rarely neutral in reaction; many are alkaline. The heating of neutral fat in a finely divided state in the presence of water and particularly if the reaction of the material is alkaline, would be expected by any chemist to result in hydrolysis or splitting of the fat. Hence, the neutral fat content of feces after drying is not always the same as before drying. This is not merely a theoretical objection but has been shown to occur in practice."

The normal values, based upon the examination of dried feces, are as follows:

	Per cent of dry weight
Total fat....	15-25
Neutral fat.....	10-15
Free fatty acids.	9-13
Combined fatty acids (soaps).....	10-15

The normal values as given by Fowweather, employing the wet method, are as follows:

	Per cent of total dry matter
Total fat...	17.5
Neutral fat.. . . .	7.3
Free fatty acids.....	5.6
Combined fatty acids (soaps).....	4.6

Fowweather lays down the following criteria for abnormal fat excretion in adults and children over two years of age:¹⁶

(a) Any specimen in which the total fat exceeds 25 per cent of the total dry matter of the feces is probably abnormal.

(b) Deficient fat digestion is suggested in any specimen in which the neutral fat exceeds 11 per cent of the total dry matter or 55 per cent of the total fat.

(c) Deficient fat absorption is suggested in any specimen in which the total fatty acid (free fatty acid plus soap) exceeds

16 per cent of the total dry matter and 75 per cent of the total fat.

Obviously, the fecal fat may consist of either unabsorbed fat, fat that has been excreted into the bowel, or both. Evidence is at hand which suggests that under normal and many abnormal conditions the fat of the feces is probably largely an endogenous excretion product. The amount excreted is quite constant under normal conditions and is independent of the diet, often remaining unchanged during periods of starvation. In the past, considerable clinical significance was attached to studies of the amount and partition of fecal fat in relation to disturbances of fat absorption and digestion. In cases of severe diarrhea, the fat content of the feces may be unusually high, due to the increased rapidity of passage of food through the bowel, with consequent impairment of absorption. Extremely large quantities of fat may be present in the feces in tropical and non-tropical sprue and in celiac disease, conditions commonly referred to as idiopathic steatorrhea.^{5,40} In these conditions the fat may constitute over 70 per cent of the dry weight of the feces, being present as both neutral fat and fatty acids, the latter usually predominating. Fatty diarrhea is usually the most constant manifestation and is accompanied by a variety of apparently secondary deficiency symptoms, including macrocytic anemia and low serum calcium and phosphorus concentrations, with evidence of rickets, osteomalacia or tetany, dependent apparently upon impaired absorption of calcium, phosphorus and vitamin D and, at times, evidence of vitamin A deficiency. There is frequently also an associated disturbance of carbohydrate metabolism, characterized by increased glucose tolerance, as evidenced by an abnormally flat blood sugar curve following the ingestion of glucose. This feature may be of value in differentiating these conditions from pancreatogenous steatorrhea, as may also be the fact that the latter condition is usually characterized by the fecal excretion of excessive quantities of nitrogen (more than 3 Gm. daily) on a fat-free diet. Similar findings with regard to fecal fat may be obtained in chronic pancreatitis or in the presence of obstruction of the pancreatic duct with the exception that in such cases the proportion of neutral fat is relatively high and that of fatty acids low. This disturbance of fat partition has been generally attributed to impaired fat digestion incident to diminution in the amount of pancreatic lipase in the intestine in disease of the pancreas (p. 493).

The feces may also contain large quantities of fat (to over 70 per cent of the dry weight) in patients with obstruction of the

common bile duct or with bile fistula. In the absence of associated marked pancreatic disturbance the relative proportion of neutral fat and fatty acids is usually essentially normal, the latter predominating. This phenomenon has been generally attributed to a disturbance of fat absorption due to the absence of bile salts from the intestine (p. 132). Recent studies^{11,36} indicate, however, that the absence of bile from the intestine causes but little impairment of fat absorption, 65 to 70 per cent of the fatty acids of the diet being absorbed in patients with bile fistula. It would appear, therefore, that, as under normal conditions, fecal fat in the absence of bile has, to a large extent, an endogenous origin.

In the light of these facts, former interpretations of variations in the amount and partition of fecal fat are open to considerable question. It seems well established that an enormous increase in the amount of fat in the feces, as in obstructive jaundice, may be due largely to increased excretion of fat rather than to diminished absorption, as was formerly believed to be the case. Moreover, since the excreted endogenous fat is in the form of neutral fat and fatty acids, it is obviously impossible to interpret alterations in the relative proportions of these substances in the feces in terms of altered digestion and absorption of ingested fat.

FAT IN URINE

Fat may appear in the urine of normal individuals following ingestion of a high fat diet (alimentary lipuria) or following administration of such substances as cod liver oil. Lipuria may also occur in association with the lipemia of diabetes mellitus and nephrosis, following fracture of long bones with injury to the bone marrow and also following extensive superficial injuries with crushing of the subcutaneous fat. It has been reported in subjects suffering from poisoning with such agents as phosphorus and alcohol. Fat in the urine may also be derived from fatty degeneration of leukocytes and epithelial cells, particularly in such conditions as pyelonephritis and nephrosis.

The terms "chyluria" and "lymphuria" are applied to conditions in which there is some obstruction to the flow of lymph in the thoracic duct, with consequent distention and rupture of the lymph vessels of the kidney or bladder, the fat content of the urine in such cases varying with the quantity of fat ingested. The urine in chyluria is definitely milky in appearance, whereas in lipuria it is rather opalescent. It must be remembered that fat appearing in the urine after catheterization

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may be due to contamination with the lubricant used in passing the catheter.

PHOSPHOLIPID METABOLISM¹¹

Phospholipids or phosphatides are found in all living matter and may be regarded as essential components of protoplasm. Three types have been identified in the animal organism: (1) lecithin, which consists of two fatty acids, glycerol, phosphoric acid and the nitrogenous base choline; (2) cephalin, which is similar to lecithin with the exception of the replacement of choline by aminoethyl alcohol; (3) sphingomyelin, which contains a fatty acid, phosphoric acid, choline and the nitrogenous base sphingosine. Each of these substances is in reality a group which contains several members differing from one another chiefly in the nature of the contained fatty acid molecule. Sphingomyelin, although present in several organs, is particularly abundant in brain and nerve tissue. From a practical standpoint, the lecithins and cephalin are the most important of the phospholipids. The cerebroside is a lipid which resembles the phosphatides in that they contain fatty acids, nitrogen and a carbohydrate group, but which differ from the latter in that they contain no phosphorus. The cerebroside, the most important of which are kerafin and phrenosin, are found particularly in brain and nerve tissue.

There is little exact knowledge regarding the functions of the phospholipids. Three main hypotheses have been advanced in this connection, outlined as follows by Sinclair: (1) They are intermediary products in fat metabolism, the replacement of the fatty acid of the fat molecule by the phosphoric acid-base complex (as in the lecithins and cephalins), together with the desaturation of the fatty acids, being a means of rendering the fatty acids readily diffusible and combustible. As noted above, there is strong evidence that phospholipids are intimately concerned with the transference of fatty acids across the intestinal membrane and with their transport to the tissues. (2) They may act as agents for the transportation of oxygen within the cells, alternately taking on and giving off oxygen at the unsaturated bonds of their fatty acid molecule. (3) According to the structural hypothesis, the phospholipids, because of their peculiar physicochemical properties, find their chief function in contributing to the constitution of protoplasm and to the construction of the cell membrane. The first of these hypotheses is the one most widely accepted at the present time.

There is also some evidence that phospholipids may play an important part in immunologic reactions. It is also well

known that cephalin is the thromboplastic element which initiates blood coagulation. This substance markedly accelerates the coagulation of blood, the phospholipid content of the blood platelets being about 12 per cent of their dry weight. No such effect is exhibited by purified lecithin.

Ingested phospholipids are probably not absorbed as such from the intestine. Under the influence of lipase they appear to be broken down into their component parts which, after absorption, undergo resynthesis to phospholipids in the body. The animal organism appears to be able to synthesize phospholipids in the absence of these substances from the diet which, however, must contain fat. These substances are excreted chiefly in the bile and the intestinal secretions, being present normally in the feces. Phospholipids have been reported in the urine of patients with albuminuria, being apparently adsorbed by the urinary protein under such circumstances. Studies of the excretion of phospholipids have little clinical significance, interest being centered particularly in their concentration in the blood, which will be considered subsequently (p. 145).

CHOLESTEROL METABOLISM^{7,8,11,12,53}

Cholesterol is an important member of the group of substances known as sterols, which are complex hydroaromatic alcohols widely distributed throughout all living matter. There is some evidence that cholesterol, or animal sterol, is in reality a mixture of two or more sterols which differ with respect to certain physicochemical and biological properties. It is now believed that the basic nucleus of the cholesterol molecule has the so-called cholane structure.⁵³ From a practical standpoint, the chief significance of this observation lies in the fact that it illustrates the important biochemical relationship between cholesterol and other physiologically important substances. Among these are the bile acids, vitamin D, certain male and female sex hormones, certain hormones of the adrenal cortex, saponin, toad poisons and carcinogenic hydrocarbons.

Little is known regarding the function of cholesterol in the organism. Many hypotheses have been advanced, among the most important of which are the following (Bills):

(1) The chemical relationship between cholesterol and the physiologically important substances enumerated above naturally raises the probability of a physiologic relationship. However, there is very little direct evidence that any such relationship exists. One important exception to this statement may be mentioned. Contrary to the formerly prevailing view, evidence has been presented that strongly suggests that vitamin D and

its provitamin in the higher animal organisms are derivatives of cholesterol and not ergosterol.

(2) Cholesterol, through the formation of cholesterol esters (combination with fatty acids), may possibly play a rôle similar to that of phospholipids in the absorption and transportation of fat.

(3) Some believe that it acts as an insulating medium for the myelin sheaths of nerves.

(4) According to some, it plays an important part in the regulation of cell permeability and membrane equilibrium.

(5) On physicochemical grounds, it has been suggested that the quantitative relationship between cholesterol and phospholipids is important in the regulation of protoplasmic structure and function. It is believed by some that cells whose contents show a high lecithin-cholesterol ratio are likely to be more permeable to water-soluble substances than those with a low ratio. Evidence has been presented that this ratio may be related to the growth and activity of cells, normal and malignant.

(6) Cholesterol neutralizes the hemolytic effects of a variety of substances, including venoms, bacterial toxins, bile salts, saponins, soaps, and so on. In the case of certain venoms the hemolytic substance counteracted by cholesterol is lysolecithin or lysocephalin and, consequently, significance has been attached to the occurrence of cholesterol and phospholipids in the tissues in a fairly constant proportion. Despite the fact that this antihemolytic action is exerted against substances foreign to the body, this property has given rise to the hypothesis that cholesterol may function as a detoxifying agent.

(7) The hypothesis has been advanced that cholesterol is concerned with the mechanism of immunologic reactions.

Cholesterol exists in the body in two states, free and esterified (combined with fatty acids). All of the cholesterol in the bile, practically all in the red blood cells, and 20 to 40 per cent of the cholesterol of the blood plasma is in the free state. It appears to be derived from both endogenous and exogenous sources, the former being fairly competent to maintain the body cholesterol within normal limits if the exogenous supply fails. Synthesis of cholesterol in the animal organism has been established beyond question, as has the fact that it can be destroyed in the body under physiologic conditions. It is now believed that cholesterol is continually being formed and destroyed in the tissues and that a positive or a negative balance may occur, depending upon whether synthesis is in excess of destruction, or vice versa.^{36,38}

When administered orally, relatively small amounts of cholesterol are absorbed in the absence of fat, bile acids also

increasing its solubility in the intestine. It seems probable that little or no absorption of plant sterols occurs in the animal organism. Cholesterol esters, after ingestion, probably must be hydrolyzed by enzymes (esterases) of the pancreatic and intestinal secretions before they are absorbed. These esters are resynthesized in their passage through the intestine before reaching the lymph stream, which is the chief channel of absorption. Absorption occurs chiefly if not entirely in the small intestine. A portion of the ingested cholesterol passes through the bowel unchanged and a portion is reduced to coprosterol through the action of anaerobic bacteria in the intestines, this substance being excreted in the feces. It is also probable that some of the cholesterol of the bile is reabsorbed in the small intestine.

There has been considerable controversy regarding the organs concerned in the synthesis and destruction of cholesterol, these processes having been attributed to the adrenals, spleen, liver, thyroid, pancreas and hypophysis. There is some evidence to suggest that cholesterol metabolism is regulated by the activity of the reticulo-endothelial system rather than by any one organ. Two divergent hypotheses have been advanced in this connection. According to one, the reticulo-endothelial cells control the normal disposition of lipoids, removing cholesterol from the blood when it is present in excess and returning it to the blood when the concentration is low. In accordance with this view, hypocholesterolemia may be due to hyperactivity of the reticulo-endothelial system and hypercholesterolemia to hypoactivity of this system, the latter condition being frequently associated with lipoidal infiltration of cells of other organs. The other hypothesis is that the reticulo-endothelial system synthesizes cholesterol and that variations in the concentration of this substance in the blood are dependent upon its variable synthesis by the reticulo-endothelial cells. As stated by Bills, when all the facts are considered, it appears likely that cholesterol, insofar as it is endogenous, originates in the cells in which it occurs.

It was formerly believed that cholesterol is eliminated chiefly in the bile. This is now known to be incorrect. It is eliminated chiefly in the intestinal secretions in the form of cholesterol and beta-cholestanol. The former is converted to coprosterol in the lumen of the intestine through the action of anaerobic bacteria, as stated above. According to Bills, the body eliminates but little cholesterol as such. Except for that which is lost in the desquamation of skin or secreted in milk, the greater part is eliminated in the feces as coprosterol and beta-cholestanol. A

little may be conjugated and pass out in the urine and some is probably completely destroyed in the organism.

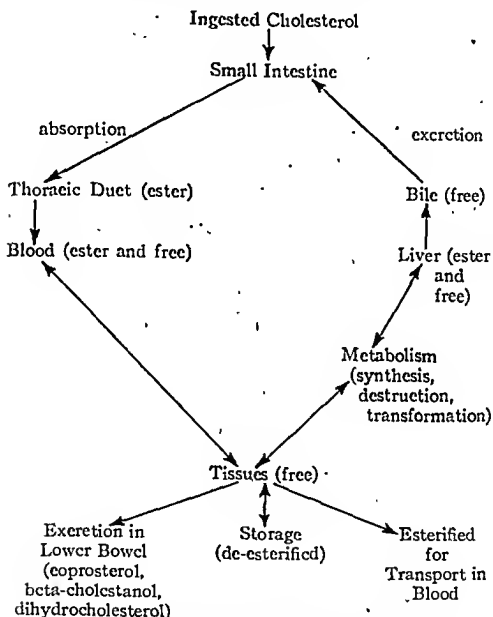


Fig. 2.—Cholesterol metabolism. (After Greene, Hots and Leahy.)¹¹

Although little is known regarding the intermediary metabolism of cholesterol, the liver appears to play an important part in this connection. This organ apparently possesses the power of actively removing cholesterol from the blood and of storing it within its substance. A high cholesterol intake in experimental animals results in a progressive increase in the quantity of this substance in the liver, other organs being relatively unaffected. As indicated below, cholesterol exists in the blood plasma in a concentration of about 140 to 250 mg. per 100 cc., about 60 to 80 per cent of the total being in the form of cholest-

terol esters, the remainder being in the free state. Since all of the cholesterol of the bile is in the free state (in humans), the liver, in addition to its function in excreting this substance, is believed by many to act as a regulator of the relationship between free cholesterol and cholesterol esters in the blood. According to this hypothesis, there is constantly occurring in the liver a reversible process of synthesis of cholesterol esters from free cholesterol and fatty acids, and of hydrolysis by means of esterases into free cholesterol and fatty acids, this process being affected in hepatic disease and in other conditions in which an increase in the proportion of free cholesterol in the blood plasma has been demonstrated (pp. 158, 429).

The subject of the intermediary metabolism of cholesterol cannot be dismissed without mention of its possible relation to the steroid hormones (ovarian, testicular, adrenal cortical) and bile acids. The intimate structural relationships between cholesterol and these substances provide strong circumstantial evidence that they may be actually derived from cholesterol and do not arise by independent biosynthesis.¹⁵

BLOOD FAT AND FATTY ACIDS^{16,17}

Reported values for the concentration of neutral fat and fatty acids in the blood vary considerably, due largely perhaps to variations in the methods employed for their determination. The concentration of total lipid in the blood of normal adults on an unrestricted diet (in the postabsorptive state) has been found to range from 400 to 1400 mg. per 100 cc. of plasma.¹⁰ This includes neutral fat, fatty acids, phospholipids and cholesterol. The neutral fat in the plasma ranges from 0 to 370 mg. per 100 cc., increasing somewhat as the total lipid concentration increases. The concentration of neutral fat in the blood cells is slightly lower than in the plasma. Considerably lower values have been obtained in preadolescent children, the average concentration below the age of five years having been reported as 182 mg. per 100 cc. in the plasma and 39 mg. per 100 Gm. of erythrocytes, and between five and ten years of age 100 mg. per 100 cc. in the plasma and 51 mg. per 100 Gm. of erythrocytes. Fatty acids in the postabsorptive state are approximately equally divided between cells and plasma. Reported values range from 190 to 450 mg. per 100 cc.¹⁴

In normal subjects the concentration of neutral fat and fatty acids in the blood plasma begins to rise one to two hours after the ingestion of fat, the maximum increase occurring within four to six hours as a rule. After the ingestion of large quantities of fat, the concentration of fatty acids in the plasma may

increase as much as 100 per cent above the resting level, returning to normal in seven to eight hours. An increase also occurs during normal pregnancy and particularly during lactation.

Although the fat content of the blood varies in certain abnormal states, determinations of the neutral fat and fatty acid concentration in the blood plasma are not commonly made clinically. This is due largely to certain technical difficulties and to the fact that alterations in plasma cholesterol, which usually occur under such circumstances, can be demonstrated much more satisfactorily. An increase in plasma fat and fatty acids usually occurs during periods of complete fasting or of maintenance on an exclusively meat diet. This increase is more marked and more consistent in subjects with large fat reserves. This phenomenon has been explained on the basis that significant increases in plasma fat occur, not necessarily when fat enters the body to be burned or conveyed to storage depots; but when there is a continuously greater demand for fat as fuel as a result of the absence of adequate amounts of available carbohydrate. An increase has also been observed during ether narcosis, in alcoholism, at times in obstructive jaundice, and following the administration of chloroform and phosphorus, the effects of the latter two agents being particularly marked in the presence of large fat reserves. The effects of ether in this connection can apparently be diminished or prevented by insulin, suggesting that the hyperlipemia in this case may be related to interference with the combustion of carbohydrate. Increase in neutral fat and fatty acids in the plasma also occurs in hypothyroidism, various forms of anemia, including pernicious anemia, hemorrhagic anemia, leukemia, hemolytic anemia, diabetes mellitus (p. 336), nephrosis and glomerulonephritis (p. 402).

Diminution in plasma fat and fatty acids appears to occur rather consistently in hyperthyroidism. It is interesting that in this condition the degree of alimentary lipemia, particularly the increase in plasma fatty acids, is greater than normal.

As stated by Peters and Van Slyke, the blood appears to serve as a transportation medium for lipids, as for other substances. Hyperlipemia may result from either diminished removal of fats from the blood or increased absorption of fats into the blood. The latter may occur as a result of increased ingestion of fat or of increased mobilization of fat from the reserve depots in the body. According to the prevailing view, as stated by these authors, hyperlipemia occurs most frequently in conditions in which the organism is forced to mobilize its fat reserves for fuel because other material, chiefly carbohydrate,

is lacking or cannot be utilized adequately; diabetes mellitus, malnutrition and fasting are examples of such conditions. There is some evidence that in diabetes mellitus the apparent increase in plasma lipids is due in part to the existing state of hemoconcentration (p. 338).

BLOOD PHOSPHOLIPIDS^{2,30,31,32}

The concentration of phospholipids in the blood is commonly expressed as either lipid phosphorus or total phosphatide, the value for the latter being obtained by multiplying the value for lipid phosphorus by the factor 23.5. Normal values for lipid phosphorus in adults in the postabsorptive state have been reported as 2.5 to 14.5 mg. per 100 cc. in the plasma and 12 to 25 mg. per 100 cc. in the red blood cells, the corresponding values for total phosphatide being 60 to 350 mg. per 100 cc. in the plasma and 300 to 600 mg. in the red cells. These values are somewhat lower in pre-adolescent children, the average phosphatide concentration in the plasma between the ages of five and ten years being 136 mg. per 100 cc. and in the red cells 244 mg. per 100 cc. An increase occurs within a few hours after ingestion of fat, the increase persisting for several hours. An increase, amounting to about 25 per cent of the nonpregnant value, occurs also in pregnancy. An additional increase has been reported during lactation, its cause being unknown.

Although apparently not of practical significance, it is interesting to note that there is considerable evidence that the phospholipids are bound in some way to the plasma proteins. Upon precipitation with ammonium sulfate, it has been found that about 30 per cent of the phospholipids of the blood plasma are carried down with the proteins. Moreover, the degree of impermeability of the capillary wall to phospholipids is of about the same order as that to plasma proteins, and there is a rough parallelism between the concentrations of protein and phospholipids in transudate fluid.²⁷

A significant increase in plasma phospholipids has been observed in diabetes mellitus, the nephrotic syndrome and chronic hemorrhage. In view of the uncertain state of knowledge regarding the intermediary metabolism and functions of the phospholipids, the causes and significance of increases in these substances in the blood cannot be stated definitely. Increases of as much as 500 per cent have been reported in patients with diabetes mellitus (p. 336), particularly in poorly nourished individuals with advanced forms of the disease. It is possible that this increase is an indication of the greater demand for the combustion of fat because of the depleted carbohydrate reserves

and the impaired combustion of carbohydrate. The phospholipid concentration usually returns to normal promptly upon restoration of normal carbohydrate metabolism following the administration of insulin.

The phospholipid increase in advanced diabetes may be dependent in part upon the existing state of malnutrition and in part upon hemoconcentration, which is a rather constant feature of this condition. The hyperlipemia accompanying the nephrotic syndrome does not appear to be accompanied by any disturbance in the absorption or utilization of fat. Individuals with this condition respond to the administration of fat with a greater increase in the fat and phospholipid concentration in the blood plasma than do normal subjects. As stated by Peters and Van Slyke, this phenomenon must therefore be dependent either upon deficient removal of these substances from the blood or upon some disturbance which causes increased liberation or mobilization of fat and phospholipids into the blood from the fat depots.

The anemia of chronic hemorrhage appears to be the only form of severe anemia accompanied by an increase in plasma phospholipids. The significance of this observation is not known. Increases have been reported occasionally in patients with epilepsy, essential hypertension, syphilis, hypothyroidism, hepatic necrosis, B-avitaminosis and Niemann-Pick's disease. The last two conditions are of particular interest, reported findings in the others being highly contradictory and apparently of little clinical significance. An increase in plasma phospholipids and fatty acids has been found to occur rather consistently in animals with vitamin B deficiency. The increase in Niemann-Pick's disease, although not always observed, is significant because of the characteristic marked increase in the phospholipid content of various organs, particularly the liver, spleen and brain. A decreased plasma phospholipid concentration has been observed in a few clinical conditions. Subnormal values are the rule in cases of pernicious anemia during relapse, an increase usually occurring promptly with the onset of a remission, whether spontaneous or induced by treatment. This decrease appears to be related to the activity of the disease process rather than to the degree of anemia, for normal and even high values may be present with severe anemia if remission has begun. Low values have also been reported, although not consistently, in cases of idiopathic hypochromic anemia, and variable findings have been reported in other forms of severe anemia with the exception of that due to acute loss of blood, in which the plasma phospholipid concentration is usually normal or increased.

Subnormal values have been obtained in patients with acute febrile infections.⁴³ The degree of such change was not constantly related to the height of the fever or the apparent severity of the infection. The phospholipid concentration rose very slowly and remained below the normal level during the convalescent period, as a rule. Similar changes could not be produced by artificial fever.

Although the practical clinical significance of this observation is not apparent, it is interesting to note that a decrease in the plasma phospholipid concentration occurs rather consistently in depancreatized dogs maintained in a satisfactory clinical condition by means of insulin. This finding, together with that of similar changes in fat and cholesterol, is in rather striking contrast to the commonly observed increase in plasma lipoids in clinical diabetes mellitus and suggests some fundamental dissimilarity between the two conditions.

Subnormal plasma phospholipid values have been reported in hyperthyroidism, but these findings do not appear to be consistent enough to be of clinical significance.

BLOOD CHOLESTEROL^{11,19,32,41}

Cholesterol exists in the blood in two forms, free and esterified (combined with fatty acids). The cholesterol content of erythrocytes is slightly lower than that of plasma or serum but, if calculated on the basis of water content, it appears to be approximately equally distributed in the water of these two media. In the red blood cells, free cholesterol constitutes about 70 to 100 per cent of the total, averaging about 85 per cent. From a clinical standpoint, interest is centered particularly upon the concentration and distribution of cholesterol in the blood plasma or serum. Normal total plasma cholesterol values obtained by different methods are extremely variable, ranging from about 120 to 350 mg. per 100 cc. The generally accepted range of normal values is 130 to 240 mg. per 100 cc. The majority of recent studies indicate that the percentage of free cholesterol appears to be a physiologic constant under normal conditions. Although Page found that this varied between 22 and 72 per cent (average 37 per cent) of the total cholesterol in a large series of normal adults, others have found the range of normal variation to be about 20 to 40 per cent.

NORMAL VARIATION IN BLOOD CHOLESTEROL

Diet. There is no unanimity of opinion regarding the influence of diet upon plasma cholesterol under normal conditions. It has been found by some that the hyperlipemia which occurs

during periods of fasting is accompanied by an increase in cholesterol, whereas others have found that prolonged starvation may lead to hypocholesterolemia. According to others, no change occurs during prolonged periods of starvation terminating fatally. Similar diversity of opinion exists with regard to the influence of fat ingestion, some observers reporting an increase, others a decrease and still others no change in plasma cholesterol concentration under such circumstances. According to Gardner and Gainsborough, the level of plasma cholesterol may be raised or lowered by prolonged feeding with high and low sterol diets, the change occurring principally in the ester fraction. According to others, however, the administration of cholesterol has no significant effect upon the plasma cholesterol concentration or partition. There is some evidence that plasma protein depletion resulting from marked restriction of dietary protein is accompanied by an increase in plasma cholesterol. It has also been found that the ingestion of large quantities of dextrose is followed by an appreciable increase in the total plasma cholesterol concentration in 60 per cent of cases, due invariably to an increase in the ester fraction, the free cholesterol remaining constant.

It seems probable that much of the discrepancy in these findings may be attributed to species differences and to variations in experimental methods. The blood cholesterol of man appears to be relatively slightly influenced by food as compared to that of other animals, particularly the rabbit and the dog. For practical clinical purposes, the influence of diet in this connection may be disregarded.

Age and Sex. The plasma cholesterol appears to be extremely low at birth (about 50 mg. per 100 cc.), increasing rapidly within the first few days of life to an average level of about 20 to 25 mg. below that of normal adults.¹⁴ This level is maintained during the period of preadolescence.

Some observers have found that the menstrual cycle is accompanied by a rather constant cyclic alteration in blood lipids, a fall in cholesterol occurring almost invariably during or within a few days of the menstrual period. This is usually preceded or followed by a blood cholesterol level definitely above the average for each individual. No significant alteration has been observed at the menopause.

Pregnancy. It is generally agreed that the plasma cholesterol increases during normal pregnancy. A maximum concentration is usually reached at about the thirtieth week, the average increase in free cholesterol at that time being about 25 per cent and that in ester cholesterol about 9 per cent. According to

some observers, the free cholesterol subsequently diminishes somewhat and the ester fraction increases slightly until an approximately normal ratio between these two fractions is attained just before parturition, at which time there is an average increase of about 25 per cent in the total cholesterol concentration. The normal level is restored about eight weeks postpartum. As stated by Boyd, none of the fifteen or more theories of the nature of the lipemia of pregnancy can be regarded as adequate at the present time. The available data indicate that it probably belongs in a group of similar lipemias represented by diabetes mellitus and experimental anemias.

HYPERCHOLESTEROLEMIA

Diabetes Mellitus. The well-recognized occurrence of lipoidemia in diabetes mellitus has attracted considerable attention (p. 336). Despite its chemical individuality, it has been shown that the concentration of plasma cholesterol in this condition tends to run approximately parallel to that of the total fatty acids of the blood, changes in the latter being reflected more or less accurately in the former. Early investigators of this problem came to the conclusion that diabetes mellitus is accompanied by a marked increase in plasma lipids, including cholesterol, and that this increase is progressive with the seriousness of the condition. Rabinowitch particularly has emphasized the importance of the plasma cholesterol concentration as an index of lipid metabolism and of prognosis in patients with diabetes. On the basis of 2000 observations on about 400 patients he concluded that the blood cholesterol is a better index of the course of the disease than is the blood sugar. Patients with normal blood sugar values following treatment, but with persistent hypercholesterolemia, were found to be more susceptible to relapse following slight dietary indiscretion or intercurrent illness, to severe complications (neuritis and gangrene) and to insulin refractoriness in the presence of complicating infectious processes. He also pointed out that responsiveness to therapy and degree of severity do not parallel one another in diabetes and that although severe diabetics may at times have a normal blood cholesterol, the latter is usually a rough gauge of the ease of control of the condition. Changes in plasma cholesterol appear to be less marked in diabetes in children. Hypercholesterolemia is observed rather consistently in the presence of diabetic coma, and cases of progressive diabetes present values that are somewhat higher than those of decreasing severity. However, normal cholesterol values are usually

obtained in uncomplicated cases in children and appear to bear no relation to the incidence of development of complications.⁵⁰

Occasionally, subnormal values for plasma cholesterol may be obtained in patients with advanced grades of diabetes. Experience has shown that this finding, which is considered elsewhere (p. 161), is of serious prognostic significance.

In the great majority of cases the hypercholesterolemia of diabetes mellitus is due to an approximately proportionate increase in both free and ester fractions. The normal ratio is usually maintained even though the total cholesterol concentration may be increased 400 to 500 per cent. There is some evidence, however, that in cases of long duration, with extensive arteriosclerosis, the proportion of cholesterol esters may be increased 10 to 15 per cent above normal. Some observers believe that this observation is of significance in connection with the possible relationship between hypercholesterolemia and arteriosclerosis.³⁴ However, at the present time there is no definite evidence of the existence of such relationship in man (p. 156).

The factors underlying the development of hypercholesterolemia in diabetes mellitus are not well understood. It may be, as suggested elsewhere (p. 144), that diabetic lipemia is an indication of a greater demand for the metabolism of fat because of the unavailability of other fuel, being similar, therefore, to the lipemia of starvation. It has been shown that hypercholesterolemia may be produced in diabetic patients by a high fat intake, undernutrition, severe acidosis and coma. Peters³² found that diabetic acidosis was accompanied by an increase in the serum protein concentration, due largely if not entirely to the associated state of dehydration, and that the progress of recovery was correlated with restoration of the normal salt and water balance and diminution in the serum protein. Other observations indicate that the factor of hemoconcentration must be considered as a possible cause of the increase in the concentration of cholesterol, fatty acids and phospholipids during diabetic acidosis. It has been found that during the acute stage of recovery the concentration of these substances decreases rapidly, the curve of cholesterol paralleling that of protein rather closely.¹⁹ Such observations suggest that hemoconcentration may be the chief cause of hypercholesterolemia in diabetic acidosis, although nutritional factors may be involved in certain instances. Although the fundamental cause of this phenomenon is not clearly understood, it is generally agreed from a clinical standpoint that lack of control of the diabetic condition is the major factor in the causation of hypercholesterolemia in diabetic patients.

Investigations in other directions reveal an intimate relationship between carbohydrate metabolism and plasma cholesterol concentration. The influence of anesthetic agents (p. 152) and of the administration of glucose (p. 148) is referred to elsewhere. The effects of insulin and epinephrine have been investigated extensively because of their profound influence upon carbohydrate metabolism. The increase in blood cholesterol incident to ether anesthesia is apparently prevented by the administration of insulin. A number of observers have reported a decrease in plasma cholesterol concentration following the injection of insulin in normal and diabetic animals and in patients with nephrosis and diabetes mellitus. However, recent studies have revealed little or no significant change following a single injection of insulin in diabetic and nondiabetic subjects, although repeated doses or prolonged insulin therapy may result in a significant diminution in the plasma cholesterol concentration. It is of interest in this connection to note that pancreatectomy in dogs is followed by a marked fall in plasma cholesterol, due to diminution in the ester fraction. This phenomenon appears to be due to the absence of some factor in the external secretion of the pancreas, for normal plasma cholesterol concentration and partition are restored by the administration of raw pancreas or pancreatic extracts (lipocaic). The effect of epinephrine upon plasma cholesterol appears to be extremely variable. No definite statement can be made regarding the influence of this factor in this connection on the basis of evidence available at the present time.

In view of the well established fact that although the absorption and oxidation of fats are unimpaired in diabetes mellitus, lipemia is rather constantly associated with that condition, it must therefore, as stated by Peters and Van Slyke, be ascribed to an abnormal localization of lipids in the blood rather than to any impairment of metabolic processes. It has been suggested that diabetic lipemia is indicative of an increased demand for the metabolism of fat because of the unavailability of carbohydrate fuel, the blood lipid content being merely representative of the quantity of that material in process of transportation. There is no definite parallelism between cholesteremia and glycemia, glycosuria, ketonuria or acidosis in diabetes. Extremely high figures have been reported, a few being above 1000 mg. per 100 cc. of plasma, several above 500 mg. and many above 300 mg. per 100 cc. The administration of insulin is usually followed by a diminution in plasma cholesterol in diabetes, but synthalin, which causes a diminution in the blood sugar concentration, apparently fails to control the hypercholesteremia. The

determination of plasma cholesterol may serve as a valuable measure of the efficacy of therapeutic procedures in diabetes and as an indication of the necessity for the continuation of insulin therapy after the restoration of the normal blood sugar level and the disappearance of glycosuria.

Anesthesia. An increase in plasma cholesterol occurs rather consistently during and following ether and, less constantly, chloroform narcosis. Ether anesthesia is accompanied by a state of hyperglycemia which frequently tends to be proportional to the degree of hypercholesterolemia. It has been found that both of these phenomena may be prevented by the administration of insulin, which suggests that the simultaneous disturbances in cholesterol and carbohydrate metabolism incident to ether anesthesia are in some way fundamentally related to one another.

The Nephrotic Syndrome. Hypcholesterolemia is a rather constant manifestation of the nephrotic syndrome (p. 402). This increase is associated with retention of other lipids in the blood, values for plasma cholesterol as high as 2200 mg. per 100 cc. having been reported and figures of 500 to 700 mg. being not unusual. Although some investigators of this problem have reported an increase in the proportion of cholesterol esters (80-90 per cent of the total) in this condition, the majority have failed to find any alteration in the distribution of cholesterol in the plasma. This hypercholesterolemia is associated with increased elimination of cholesterol in the urine and with its deposition in the renal tubular epithelium. An increase in plasma cholesterol is also found occasionally in cases of acute glomerulonephritis.

The significance of this phenomenon and the factors responsible for its development are not clearly understood. Its association with a diminished concentration of serum protein and particularly serum albumin suggests the possibility that both phenomena may be concomitant if not related features of some underlying metabolic disorder. It has been suggested that the lipidemia of the nephrotic syndrome may represent an attempt to maintain the osmotic pressure of the blood plasma which is lowered as a result of the diminution in serum protein (p. 403). This explanation, however, appears to be unsatisfactory. Although edema, hypoproteinemia and hypercholesterolemia are usually present concomitantly in this condition, there is no constant quantitative relationship between the degree of cholesterolemia and the other two phenomena. Findings in animals with experimentally produced nephrotic edema fail to support the hypothesis of a fundamental relationship between the decrease in plasma protein and the increase in cholesterol. In fact, a

diminution in plasma cholesterol may occur under such circumstances.

Some attribute the hypercholesterolemia of the nephrotic syndrome to some defect in the mechanism which ordinarily removes cholesterol from the blood or to some disturbance which causes excessive mobilization of lipids from the fat depots. It may depend upon diminution in the activity of the reticulo-endothelial system in removing cholesterol from the blood, resulting from alteration in the colloidal state of the plasma due to diminution in its protein content. Although an increase in other plasma lipids is observed commonly in all types of chronic glomerulonephritis, hypercholesterolemia occurs usually only in the presence of the nephrotic syndrome complicating the nephritic process. In the absence of this syndrome, the plasma cholesterol concentration is usually within normal limits in patients with chronic glomerulonephritis. Distinctly sub-normal values may be obtained in patients in the terminal stages of this condition (p. 160).

Hepatic and Biliary Tract Disease.^{11,13,19} The plasma cholesterol concentration is usually increased in jaundice due to uncomplicated common duct obstruction, the degree of hypercholesterolemia roughly paralleling that of hyperbilirubinemia and returning to normal with release of the obstruction. In about 50 per cent of such cases the cholesterol esters increase concomitantly with the total cholesterol, the normal ratio between the free and the ester fractions being maintained; in the remainder, the increase occurs practically entirely in the free fraction. This has been attributed to interference with the absorption of fat and cholesterol esters from the intestine as a result of the absence of bile. However, these findings and their interpretation are contradicted by the findings of Epstein and Hawkins, who found an elevation of cholesterol esters in the plasma in the great majority of cases of common duct obstruction and bile fistula in which little or no bile was present in the intestine. Moreover, as stated elsewhere (p. 137), there appears to be little if any impairment of fat absorption under such circumstances.

The cause of the increase in plasma cholesterol in obstructive jaundice is not clear. The former view that it is a retention phenomenon was based on the belief that cholesterol is excreted chiefly by the liver. This is now known to be erroneous, the intestinal mucosa being the chief site of cholesterol excretion. This view is also contradicted by the observation of an increase in plasma cholesterol in cases of bile fistula. It would appear that

this phenomenon is related in some way to the absence of bile or certain of its constituents from the intestine.

Patients with jaundice of extrahepatic obstructive origin not infrequently present normal or even subnormal plasma cholesterol values. Such findings are usually dependent upon the presence of some complicating factor, among the most common of which are cachexia, infection, superimposed hepatocellular damage or terminal cholemia. The occurrence of hypocholesterolemia in these conditions is discussed elsewhere (pp. 158-160). Under such circumstances, the cholesterol ester fraction is abnormally low and the ratio of esterified to free cholesterol is decreased. When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance, since it usually indicates the presence of one or more of the complicating factors enumerated above.

Hypercholesterolemia occurs at times in cases of mild hepatocellular jaundice (hepatitis), but much less frequently than in obstructive jaundice. When it does occur, it is usually dependent upon an increase in the free cholesterol fraction, the ester cholesterol-free cholesterol ratio being diminished. Except in the terminal stages, plasma cholesterol concentration and partition are normal in portal cirrhosis and are either normal or insignificantly elevated in cholecystitis or cholelithiasis without biliary obstruction. The hypothesis of a relationship of cause and effect between hypercholesterolemia and cholelithiasis has not been substantiated by recent investigations. Experimental studies indicate that there is no constant relationship between the concentration of cholesterol in the bile and in the blood plasma, and it would appear that the formation of gallstones is probably dependent largely upon factors operating within the extrahepatic bile passages.

Myxedema.^{18,22,51,52} Myxedema is rather constantly associated with an increase in blood lipids, the degree of hypercholesterolemia being roughly proportional to the diminution in the basal metabolic rate. Values above 600 mg. per 100 cc. have been reported in both adults and children. There is evidence that this increase is dependent upon hyperactivity of the anterior hypophysis secondary to the diminished thyroid function, since it does not develop in hypophysectomized-thyroidectomized animals. A return to normal usually follows the administration of thyroid extract. Estimation of the plasma cholesterol concentration may be of value in the diagnosis and regulation of thyroid dosage in the treatment of children with hypothyroidism in whom the determination of the basal metabolic rate may not be practicable. However, although it tends

to be elevated, at times markedly (above 500 mg. per 100 cc.), the cholesterol concentration in hypothyroid children has been found to fluctuate within wide limits upon repeated determination⁵² and to remain within normal limits in some cases of severe hypothyroidism. This may be due to variation in pituitary activity. Decrease in plasma cholesterol during thyroxin therapy and a marked increase following cessation of therapy has been found to be of considerable diagnostic significance in children.^{51,52}

Xanthomatosis.⁴⁷ Xanthomas, particularly if present in large numbers (multiple xanthomas), are often associated with hyperlipemia. Because of this fact the condition is frequently encountered as a complication of advanced diabetes mellitus, long-standing obstructive jaundice or lipid nephrosis. Under such circumstances extremely high blood cholesterol values may be observed. Rarely, multiple xanthomas may occur without hypercholesterolemia but with an increase in total blood fat. Occasionally the condition, with hypercholesterolemia, is encountered in the absence of diabetes, jaundice, lipid nephrosis or any other demonstrable cause for the existing lipidemia (idiopathic hypercholesterolemia). Such cases may be dependent upon some fundamental disturbance of the reticulo-endothelial system. Hypercholesterolemia may occur in the symptom complex known as Hand-Schüller-Christian disease, which is believed to represent a disturbance of lipid metabolism and which constitutes one of the several conditions included under the designation of xanthomatosis or lipid granulomatosis. Cholesterol constitutes about 50 per cent of the total lipid content of the xanthomatous lesion in this condition. The increased lipid content of the characteristic lesions in Niemann-Pick's disease is due chiefly to an increase in sphingomyelin and in Gaucher's disease to an increase in cerebroside (kerasin), these conditions representing other forms of xanthomatosis. In Tay-Sachs disease, the abnormal lipid deposits consist largely of a substance (Substance X) containing sphingosine, lignoceric acid, galactose and neuramic acid, differing from kersasin (Gaucher's disease), which does not contain the latter molecule (neuramic acid). In many cases of Hand-Schüller-Christian disease the plasma cholesterol is within normal limits. According to some observers hypercholesterolemia occurs during the active stages of the disease, normal values being obtained during periods of apparent quiescence.

Thannhauser regards essential xanthomatosis as a cellular disease of reticular cells caused by an intracellular disorder of their cholesterol metabolism. He suggests the following classification of xanthomatous diseases, which has a direct bearing upon

the occurrence or nonoccurrence of hypercholesterolemia in these conditions:

I. Primary essential xanthomatosis.

- A. Xanthomatous lesions of the bile ducts, from xanthomatous
4. Xanthomatous lesions of the bile ducts.
 5. Xanthomatous lesions of the bile ducts.
 6. Xanthomatous lesions of the bile ducts.

glands (these may be present in group B).

B. Normocholesterolemic type.

1. Xanthomata disseminata of the skin.
2. Osseous xanthomata of the skull, scapula, pelvis, orbit and extremities.
3. Xanthomata of the hypophysis and tuber cinereum with diabetes insipidus. Also of the brain and dura.
4. Xanthomata of the lung and pleura, with pulmonary fibrosis.
5. Scattered nests of xanthoma cells in the liver, spleen and lymph glands (these may be present also in group A).

II. Localized xanthoma cell formation in true tumors.

Acute Hemorrhage. Whereas the plasma cholesterol concentration is often low in patients with various other forms of severe anemia, high values may be obtained following acute hemorrhage. This increase may be pronounced within forty-eight hours after the acute loss of blood and may persist for several days. The cause of this increase in plasma cholesterol is not known. The suggestion has been advanced that it may be due to the loss of plasma protein, representing an attempt to maintain the colloid osmotic pressure of the plasma at a normal level. There is little evidence to substantiate this hypothesis. It may be due either to decreased oxidation resulting from the diminution in erythrocytes or to excessive mobilization of lipids from the fat reserves in the tissues.

Vitamin A Deficiency. An increase in plasma cholesterol has been observed in experimental animals with vitamin A deficiency. Because of lack of sufficient data, no statement can be made at the present time regarding the significance of such findings in clinical states of vitamin A deficiency.

Atherosclerosis. Despite numerous attempts to demonstrate that hypercholesterolemia plays an important part in the development of atherosclerosis, such a relationship has not been satisfactorily established.^{22,35} Several authorities, however, believe that the hypercholesterolemia of diabetes mellitus is a factor of fundamental importance in contributing to the development of arteriosclerosis in patients with this condition.^{24,33,34}

Nevertheless, critical analysis of findings in large numbers of patients with atherosclerosis fails to reveal any significant consistent abnormality of plasma cholesterol concentration or partition in the absence of such conditions as diabetes and nephrosis. According to Bruger, the development of degenerative diseases such as diabetes mellitus, arthritis and arteriosclerosis is as a rule followed, and not preceded, by hypercholesterolemia. He believes that the increase in plasma cholesterol in these conditions should be regarded as a complication rather than as an etiologic factor.

Miscellaneous Conditions. An increase in plasma cholesterol has been observed occasionally in patients with hypertrophic osteoarthritis, senile cataract, psoriasis and dermatitis, and in infants with celiac disease. The designation "idiopathic familial lipemia" has been applied to a condition of unknown etiology.²¹ There is a familial incidence and a rather characteristic clinical picture of splenomegaly, hepatomegaly, and periodic attacks of acute abdominal pain, during which the blood lipids fall. In one case, the total serum lipids were as high as 8.2 Gm. per 100 cc., the cholesterol being 398 mg. and the lipid phosphorus 19.4 mg., the increase being due principally to neutral fat.

HYPOCHOLESTEROLEMIA

Anemia. The plasma cholesterol is uniformly low in pernicious anemia during relapse, and in hemolytic jaundice, values as low as 50 mg. per 100 cc. having been reported. Hypocholesterolemia is likewise observed in practically all patients with severe hypochromic anemia with the exception of severe aplastic anemia and anemia following acute hemorrhage. In severe hypochromic anemias and also in pernicious anemia and in hemolytic jaundice the diminution in plasma cholesterol is associated with reduction in plasma phosphatide and an increase in fat (p. 144), these changes occurring typically with hemoglobin values below 50 per cent but bearing no direct relation to the degree of anemia. In typical cases of pernicious anemia in relapse the cholesterol rises suddenly at the onset of remission, whether spontaneous or induced by treatment. This occurs simultaneously with the reticulocyte response and occurs usually before any increase in hemoglobin or red blood cells. Consequently, as emphasized by Muller, normal or even elevated plasma cholesterol values may be present in patients with severe anemia, provided that remission has begun. This may account for certain of the discrepancies in reported observations of plasma cholesterol concentration in this condition.

Hepatic Discasc.^{11,12,20} Hepatocellular damage is frequently accompanied by a diminished proportion of cholesterol esters in the blood plasma (normally 60-80 per cent of the total), constituting the so-called "Estersturtz" of Thannhauser and Schaber. This may not be, but usually is, associated with a diminution in total cholesterol concentration. The chief practical significance of this observation lies in the fact that it offers some hope of differentiating hepatocellular jaundice from simple obstructive jaundice, in which hypercholesterolemia is the rule (pp. 153, 428). Hypocholesterolemia, with a diminished proportion of cholesterol esters, has been observed in cases of hepatocellular damage due to the following causes: (a) drugs, such as arsphenamine preparations, cinchophen, phenobarbital, chloroform, carbon tetrachloride and phosphorus; (b) pneumonia, myocardial failure, yellow fever and spirochetal jaundice; (c) catarrhal jaundice, toxic hepatitis, and acute, subacute and chronic diffuse necrosis of the liver. Similar findings have been obtained in experimental animals.

A pronounced discrepancy between the degree of bilirubinemia and of cholesterolemia is usually observed in degenerative conditions of the liver (hepatocellular damage); the more severe the hepatic damage the greater the tendency toward hypocholesterolemia. This discrepancy between the hyperbilirubinemia and cholesterolemia is of value in differentiating between purely mechanical obstructive lesions and superimposed or primary degenerative lesions of the hepatic parenchyma. In parenchymatous disease of the liver, a drop in the ester fraction often parallels the severity of the hepatic damage, and in rapidly fatal cases this fraction may be low or absent throughout the course of the disease. In the event of improvement the initially low ester values eventually increase. Low values may also be obtained in terminal stages of portal cirrhosis. When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance, since it is indicative usually of extensive superimposed hepatocellular damage.

Several hypotheses have been advanced in explanation of the fall in cholesterol esters in hepatic disease. Among these are faulty absorption from the intestine, impaired esterification in the liver and storage of esters in the liver. There is some evidence that bile salts, which influence resynthesis and hydrolysis of esters by enzymes (esterases), may play an important part in this connection. It has been found that increasing concentrations of bile salts inhibit the esterification of free cholesterol. However, the application of this observation in hepatic disease is not

readily apparent. The fact that hypocholesterolemia with a diminished proportion of cholesterol esters occurs also in other conditions unrelated to hepatic disease suggests the operation of some more fundamental mechanism in the causation of this phenomenon.

Infection.⁴²⁻⁴⁶ Hypocholesterolemia and a diminution in the concentration of other blood lipids is observed frequently during the course of acute infectious diseases. The most marked decrease usually occurs in the ester fraction which, together with the total cholesterol concentration, rises to normal and sometimes supernormal levels during convalescence. Similar changes occur simultaneously in fatty acids and phospholipids. There appears to be no constant relationship between the degree of hypocholesterolemia and the severity of the clinical manifestations. Such changes cannot be produced by the induction of artificial fever. According to some observers, hypocholesterolemia is of prognostic significance in tuberculosis, in which condition it appears to be related to the severity of the process. The cause of these changes in plasma cholesterol in infectious diseases is not understood, but they may be related in some way to the phenomenon of immunity. This is suggested by the observation that the fall in temperature in pneumonia induced by administration of specific serum is followed promptly by increase in cholesterol (and fatty acids) whereas similar fall in temperature and clinical improvement induced by sulfonamide therapy are followed by a significant increase in cholesterol only after an interval of four to seven days.⁴⁵ This corresponds approximately to the time at which type-specific antibodies appear in the blood in sulfonamide-treated cases.

Hyperthyroidism.²² In thyroid disease, there appears to be a roughly reciprocal relationship between basal metabolic rate and plasma cholesterol concentration. Low values (60-100 mg. per 100 cc.) may be obtained in patients in or near thyroid crises. The average values in exophthalmic goiter are somewhat lower than in toxic nodular goiter. The apparent absence of such changes with increased basal metabolic rates due to administration of dinitrophenol suggests that the changes in plasma cholesterol in thyroid disease are not directly related to the metabolic rate but to other actions of thyroid secretion.

Diminution in the basal metabolic rate in patients with hyperthyroidism following administration of iodine or subtotal thyroidectomy is accompanied by an increase in plasma cholesterol concentration. On the basis of a large series of observations, Hurxthal arrived at the following conclusions: (a) If the plasma cholesterol concentration is below 100 mg. per 100 cc. in

toxic goiter, in the absence of acute infection, the patient is almost certainly very ill. (b) Cholesterol values above 180 mg. per 100 cc. indicate only slight or moderate toxicity. (c) The determination of plasma cholesterol concentration appears to be of little diagnostic value in borderline cases, except that if it is above 200 mg. the condition is not likely to be hyperthyroidism unless there has been a complete remission due to the administration of iodine.

Determination of plasma cholesterol is of little clinical value in hyperthyroidism. The nutritional status exerts a considerable influence and, whereas the plasma lipid level is closely related to the degree of thyroid activity in uncomplicated cases, the problems of diagnosis and prognosis in such cases are relatively simple and are not usually further clarified by investigation of the plasma cholesterol concentration. Moreover, the latter has been found to be no criterion of the response of the patient to thyroidectomy or iodine therapy.²¹

Inanition. A diminution in plasma cholesterol is commonly observed in conditions accompanied by wasting and cachexia. Its significance in these conditions is not clearly understood. In many cases there is an associated decrease in plasma protein concentration, both of these phenomena apparently being directly related to the state of malnutrition.

Terminal States.^{4,12} The frequent occurrence of hypercholesterolemia in the nephrotic syndrome has been referred to elsewhere (p. 152). In non-nephrotic forms of glomerulonephritis, however, although the total fat, fatty acid and phospholipid concentrations in the plasma are commonly increased, plasma cholesterol is frequently normal or subnormal, this dissociation suggesting, as stated by Peters and Van Slyke, a functional differentiation between these lipid fractions. The variability of the plasma cholesterol in chronic glomerulonephritis has attracted considerable attention, practically all investigators of this problem coinciding in the opinion that a fall from a previously elevated or normal level is of serious prognostic significance, particularly if associated with increasing nitrogen retention. There usually appears to be a roughly reciprocal relationship between these two factors in the terminal stages of this disease, although there is no constant quantitative relationship between the degree of cholesterolemia and of nitrogen retention.

Low plasma cholesterol values (50–100 mg. per 100 cc.) have also been obtained in patients in the terminal stages of a variety of diseases in the absence of nitrogen retention.¹² Among these are arteriosclerosis, congestive heart failure, carcinoma, acute

pancreatitis, pulmonary tuberculosis, bacterial endocarditis and other forms of bacteremia, coronary artery occlusion and diabetes mellitus.

No definite statement can be made at the present time regarding the cause of hypocholesterolemia in terminal states. Some believe that it is due to anemia and cachexia, which are frequently present in such patients. However, these factors are frequently absent in those dying of peritonitis, pneumonia, coronary artery occlusion, congestive and left sided heart failure and in certain patients with urinary obstruction due to prostatic enlargement. If one is to assume that the mechanism underlying the production of hypocholesterolemia in various terminal states is essentially the same in each case, it would appear that some other explanation must be sought. There is some evidence that this mechanism involves an increased rate of removal of cholesterol from the blood as a result of a state of increased reticulo-endothelial cell activity. Whatever may be the cause, the development of hypocholesterolemia under such circumstances is of serious prognostic significance, especially in diabetes mellitus and chronic glomerulonephritis, in which the plasma cholesterol may previously have been elevated.

Miscellaneous. Hypocholesterolemia has been observed in patients with prostatic obstruction and with intestinal obstruction, its occurrence in these conditions being of rather serious prognostic import and indicative of poor defensive powers. Low plasma cholesterol values have also been reported during epileptic seizures and in arthritis.

KETOSIS (see p. 336)

Ketosis is the term applied to the accumulation of excessive quantities of ketone bodies (aceto-acetic acid, beta-hydroxybutyric acid, acetone) in the body and their excessive elimination in the urine. Under normal conditions these substances are present in the blood in concentrations of 1.5 to 2.5 mg. per 100 cc., expressed as acetone. On a mixed diet, less than 1 Gm., expressed as beta-hydroxybutyric acid, is eliminated in the urine of normal individuals in twenty-four hours, if sufficient amounts of carbohydrate are ingested. Ketone bodies are derived from fatty acids having an even number of carbon atoms, and their formation is commonly regarded as the result of incomplete oxidation of such fatty acids. According to the prevailing concept, as carbohydrate utilization diminishes and the combustion of fat correspondingly increases, a point is eventually reached at which the oxidation of fatty acids becomes impaired or incomplete. A quantitative relationship has been

established between glucose utilization and fatty acid oxidation. It is believed that 1 Gm. of glucose must be utilized in the tissues in order to effect the complete oxidation of 1.5 Gm. of fatty acid. This fatty acid-glucose ratio is termed the ketogenic-antiketogenic ratio. If this ratio exceeds 1.5, fatty acid oxidation is impaired and stops at the 4-carbon-atom stage, with the accumulation of excessive quantities of aceto-acetic acid. Beta-hydroxybutyric acid is formed from this substance by reduction and acetone by the liberation of CO_2 .

As suggested above, the prevailing view regarding the action of carbohydrate in diminishing or preventing ketosis is that it accelerates the utilization of ketone bodies and facilitates the oxidation of fatty acids beyond the stage represented by the formation of these substances. Recent studies have cast doubt upon the validity of this hypothesis and have demonstrated additional facts regarding the site of formation of ketone bodies and factors influencing their production.

There is strong experimental evidence that ketone bodies are formed only in the liver. Consequently, the antiketogenic effects of glucose and insulin cannot be related to the utilization of carbohydrate in the tissues generally, but must be due to an effect produced by these agents in the liver. It is generally recognized that only glycogenic substances are antiketogenic and that the glycogen content of the liver is diminished in conditions associated with excessive accumulation of ketone bodies. Mirsky believes that a decrease in liver glycogen is probably the essential stimulus to ketone formation by the liver and that any agent that inhibits excessive breakdown of liver glycogen also prevents the formation of ketone bodies. He regards the latter as normal end-products of fat metabolism in the liver. As the glycogen content of the liver cells diminishes, catabolism of fat in the liver increases, with the production and accumulation of excessive quantities of ketone bodies (Fig. 3).

A ketogenic principle has been demonstrated in extracts of the anterior lobe of the hypophysis, the administration of which results in the excessive accumulation and excretion of ketone bodies in experimental animals under a variety of conditions.² There is some evidence that the presence of the adrenal cortex is necessary for the production of this effect.²⁵ There is evidence also that this principle acts solely on the liver, the resulting ketosis being associated with marked depletion of liver glycogen. It does not appear to stimulate the oxidation of fat in extra-hepatic tissues.

The concept of ketosis suggested by these observations may be summarized as follows:^{28,29} Ketone bodies are in themselves

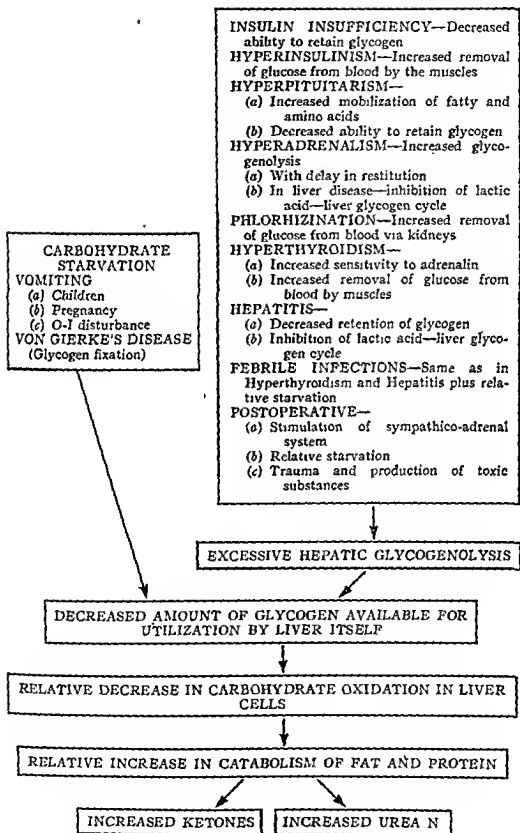


Fig. 3.—Summary of conditions in which ketones are produced in excess indicating the nature of the mechanism involved. (After Mirsky, I. A.: *J. Med.* 18: 222, 1937.)

end-products of fat metabolism in the liver. This organ preferentially burns carbohydrate; in the absence of adequate quantities of carbohydrate, fat and protein are burned in excess. When this occurs, ketone bodies, as end-products of fat metabolism in the liver, pass into the blood stream and are utilized in the extrahepatic tissues. The degree of resulting ketonemia and ketonuria depends upon the difference between the rate of ketone formation and the rate of ketone utilization. According to this hypothesis, instead of facilitating fat metabolism or its combustion, glucose actually inhibits it.

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Chapter IV

Calcium Metabolism

ABSORPTION AND ELIMINATION^{7,29,30,31,34}

CALCIUM is absorbed chiefly in the upper portion of the small intestine, the degree of absorption being governed chiefly by three factors: (1) the hydrogen ion concentration in the intestine, (2) other substances in the diet and (3) vitamin D. Since calcium salts, particularly the phosphate and carbonate, are quite soluble in acid solutions and are relatively insoluble in alkaline solutions, it is evident that factors which tend to increase intra-intestinal alkalinity will proportionately diminish the degree of absorption of calcium and, vice versa, factors which increase intra-intestinal acidity will correspondingly increase calcium absorption. Under conditions of normal gastric acidity, compounds of calcium with weak organic acids are converted to the soluble chloride and, if retained in the stomach for a sufficient period, even the less soluble basic phosphate may go into solution. However, solution of calcium salts by the gastric juice is not essential, as absorption may occur in achlorhydria, or after gastrectomy or administration of alkali. The acidity in the duodenum is of considerable importance, ranging normally from pH 2.3 to 7.0. This factor largely determines whether most of the calcium is in the form of the acid or the basic phosphate and, since the former is the more soluble, a higher acidity tends to facilitate absorption of calcium. Calcium chloride and acid phosphate are probably absorbed from the duodenum before the gastric juice acidity is neutralized and, subsequently, absorption of calcium may be favored by the formation of organic acids (carbonic and lactic).

If the phosphate-calcium ratio in the upper intestine is excessively high, calcium absorption is diminished due to the production of an unduly large quantity of insoluble tertiary calcium phosphate. This is particularly true if calcium is supplied in insufficient amounts. A similar inhibiting effect is apparently exerted by an excess of magnesium and of potassium. Disturbances of fat absorption and increased fat excretion act in the same manner since the presence of large amounts of fatty acids in the intestine results in the formation of calcium soaps which, being insoluble, are not absorbed and are eliminated in

the feces. This condition is observed clinically in obstructive jaundice, sprue, celiac disease and similar disorders. Diminished absorption of calcium under these circumstances may be due in part also to interference with absorption of vitamin D. Inadequate absorption may also result from protracted diarrhea, due to the rapid passage of intestinal contents through the bowel, and to gastrocolic fistula.

It is believed by some that the chief effect of the antirachitic factor (vitamin D) upon calcium metabolism is to increase the absorption of calcium from the intestinal tract. It appears possible, however, that the main activity of the antirachitic factor may be exerted in the intermediary metabolism of phosphorus and calcium (p. 173).

The skeleton is the normal storehouse of calcium in the body, relatively little being present in the soft tissues. In view of the present knowledge regarding certain phases of intermediary calcium metabolism it is evident that the calcium and phosphorus deposits in the skeleton may be readily mobilized under physiologic and pathologic conditions and, from a metabolic standpoint, are comparable to the deposit of glycogen in the liver. The details of the intermediary metabolism of calcium are not clearly understood; the influence of the parathyroid hormone and the antirachitic factor, both of which undoubtedly play an important part in this connection, will be discussed below.

Normal adults are in a state of calcium equilibrium. If, however, a normal individual is maintained upon a diet containing an extremely small amount of calcium, a negative balance is produced. Calcium is excreted by the kidneys, the liver and the epithelium of the large bowel. The fact that the fecal calcium includes also that portion of the ingested calcium that has escaped absorption and has passed through the gastro-intestinal tract, has rendered difficult the exact quantitative determination of intestinal excretion of calcium. With low and moderate levels of intake (0.1-0.5 Gm. daily), about 30-50 per cent is eliminated in the urine, while with high levels of intake (1.0 Gm.) about 10-25 per cent is so eliminated. However, there is considerable deviation from these values, due probably to variable dietary, metabolic and gastro-intestinal factors. The renal "threshold" for excretion of calcium probably lies between 6.5 and 8.5 mg. per 100 cc. of serum, little being eliminated in the urine at lower serum calcium concentrations.² This threshold is raised in the presence of renal functional impairment, the urinary calcium constituting a steadily diminishing fraction of the total excretion in progressive kidney damage.

BLOOD CALCIUM^{7,8,22,24}

The consensus of opinion is that there is no calcium in the red corpuscles. Since calcium is contained entirely in the plasma it is obvious that the calcium content of whole blood will vary inversely as the corpuscular volume. Because of the great variability of the latter factor, estimations of the calcium content of whole blood are valueless from a practical standpoint. The calcium content of human serum ranges from 8.5 to 11.5 mg. per 100 cc. During infancy and early childhood (up to about twelve years) the average values approach the upper limit of this range, subsequently falling with advancing years. Fetal blood serum at term contains 11-12 mg. per 100 cc., the maternal serum calcium being usually 8.5-9.5 mg. The slight fall during the last month of pregnancy has been attributed to the heavy drain on the mineral reserves of the maternal organism, but it seems likely that endocrine factors participate in the production of this phenomenon.

Calcium exists in the blood, probably as a phosphate, in much higher concentration than would be possible in distilled water. Its solubility in plasma is dependent largely upon the following factors: (1) the pH, (2) the CO₂ tension, (3) the protein concentration, (4) the total ionic strength, (5) the magnesium concentration and (6) the inorganic phosphate concentration. Their influence may be illustrated as follows:³⁰ If 10 mg. of calcium and 4 mg. of phosphorus are placed in 100 cc. of distilled water (pH 7.0), only a small amount of the resulting salt will go into solution, but more will be dissolved at the pH of serum than in a more alkaline solution. At a constant pH its solubility is inversely proportional to the concentration of bicarbonate and phosphate ions, and at constant hydrogen, bicarbonate and phosphate ion concentrations the solubility is increased by addition of other soluble ions in amounts present in normal plasma. The quantity of calcium still undissolved may be brought into solution by addition of 1 to 3 mg. of magnesium and 4 to 5 Gm. of albumin per 100 cc. From a practical standpoint, the effects of alteration in phosphate and protein concentrations are of greatest importance.

Serum calcium consists of two physiologically distinct fractions which have been termed diffusible and nondiffusible. The diffusible fraction is that portion of the serum calcium which is capable of passing through artificially prepared membranes and, presumably, through the living capillary wall and cell membrane; the nondiffusible fraction normally cannot, owing probably to its combination with serum proteins, particularly albumin.

Under certain conditions (addition of large amounts of calcium or phosphorus, large doses of parathyroid hormone or vitamin D), the nondiffusible fraction may possibly include a colloidal complex of calcium phosphate, but it usually consists entirely of calcium proteinate. The diffusible portion, which probably is or contains the physiologically active calcium fraction, normally ranges from 4.5 to 6 mg. per 100 cc., constituting from 40-60 per cent of the total serum calcium; the diffusible fraction of the serum calcium may be determined by dialysis or ultrafiltration through an artificial membrane. Although the two fractions (diffusible and nondiffusible) may vary independently of each other under certain circumstances, there is evidence that they are in a state of rather unstable equilibrium, which is probably controlled to a certain extent by the parathyroid hormone.¹¹

The previously generally accepted view was that only a small portion of the total serum calcium exists in ionized form. The concentration of calcium ions in the blood was believed to be dependent upon several factors, the most important of which were the hydrogen ion, bicarbonate, and phosphate ion concentrations. The relationship has been expressed by Freudenberg and György by means of the following formula:

$$\frac{\text{Ca}^{++}(\text{HCO}_3)^-(\text{HPO}_4)^{--}}{c\text{H}^+} = K$$

It may be seen that the concentration of calcium ions decreases as the bicarbonate and phosphate ion concentrations increase and as the hydrogen ion concentration decreases, and vice versa. Kugelmass and Shohl proposed the following equation as a means of expressing the amount of ionic calcium which is apt to be present for saturation under the existing conditions:

$$\text{Ca}^{++} = \frac{7.6 \times 10^{-5}(\text{H}^+)}{\text{HCO}_3^-(\text{HPO}_4)^{--}}$$

According to their calculation the blood serum contains approximately 2 mg. of ionized calcium per 100 cc. If it be assumed that the ionized fraction alone produces a pharmacologic calcium effect it is obvious that according to this concept only a small portion of the total serum calcium is in this active form. It is probable that all of the ionized calcium is diffusible since the evidence at our disposal indicates that the nondiffusible calcium is in a nonionized form. On this basis, the quantitative relationship between the various calcium states in the blood serum may therefore be expressed as follows:

BLOOD CALCIUM^{7, 8, 29, 34}

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Serum calcium consists of two physiologically distinct fractions which have been termed diffusible and nondiffusible. The diffusible fraction is that portion of the serum calcium which is capable of passing through artificially prepared membranes and, presumably, through the living capillary wall and cell membrane; the nondiffusible fraction normally cannot, owing probably to its combination with serum proteins, particularly albumin.

Under certain conditions (addition of large amounts of calcium or phosphorus, large doses of parathyroid hormone or vitamin D), the nondiffusible fraction may possibly include a colloidal complex of calcium phosphate, but it usually consists entirely of calcium proteinate. The diffusible portion, which probably is or contains the physiologically active calcium fraction, normally ranges from 4.5 to 6 mg. per 100 cc., constituting from 40-60 per cent of the total serum calcium; the diffusible fraction of the serum calcium may be determined by dialysis or ultrafiltration through an artificial membrane. Although the two fractions (diffusible and nondiffusible) may vary independently of each other under certain circumstances, there is evidence that they are in a state of rather unstable equilibrium, which is probably controlled to a certain extent by the parathyroid hormone.¹¹

The previously generally accepted view was that only a small portion of the total serum calcium exists in ionized form. The concentration of calcium ions in the blood was believed to be dependent upon several factors, the most important of which were the hydrogen ion, bicarbonate, and phosphate ion concentrations. The relationship has been expressed by Freudenberg and György by means of the following formula:

$$\frac{\text{Ca}^{++}(\text{HCO}_3)^-(\text{HPO}_4)^{--}}{c\text{H}^+} = K$$

It may be seen that the concentration of calcium ions decreases as the bicarbonate and phosphate ion concentrations increase and as the hydrogen ion concentration decreases, and vice versa. Kugelmass and Shohl proposed the following equation as a means of expressing the amount of ionic calcium which is apt to be present for saturation under the existing conditions:

$$\text{Ca}^{++} = \frac{7.6 \times 10^{-5}(\text{H}^+)}{\text{HCO}_3^-(\text{HPO}_4)^{--}}$$

According to their calculation the blood serum contains approximately 2 mg. of ionized calcium per 100 cc. If it be assumed that the ionized fraction alone produces a pharmacologic calcium effect it is obvious that according to this concept only a small portion of the total serum calcium is in this active form. It is probable that all of the ionized calcium is diffusible since the evidence at our disposal indicates that the nondiffusible calcium is in a nonionized form. On this basis, the quantitative relationship between the various calcium states in the blood serum may therefore be expressed as follows:

Total serum Ca = Diffusible Ca + Nondiffusible Ca		
9-11.5 mg.	4.5-5.5 mg.	4.5-6 mg.
	(1) Ionized 2 mg.	Nonionized
	(2) Nonionized	

On the basis of ultrafiltration and adsorption experiments, employing 40 per cent dry barium sulfate as an adsorbent, Benjamin and Hess postulated the presence of at least four physicochemical states of calcium in normal blood serum, two diffusible and two nondiffusible: (1) an adsorbable calcium-phosphorus complex, constituting about two thirds of the diffusible fraction; (2) calcium ion, constituting the remainder of the diffusible fraction; (3) an adsorbable, nondiffusible calcium-phosphorus complex, constituting about one fourth of the nondiffusible fraction; (4) protein-bound calcium, constituting the remainder of the nondiffusible fraction. Although the concept of a colloidal calcium-phosphorus complex is supported by evidence reported by a number of observers, the partition of serum calcium into four fractions on the basis of adsorption by barium sulfate is open to question.

McLean and Hastings have developed a biological method for the estimation of the concentration of calcium ions, in which the isolated frog heart is employed as an indicator. The following conclusions were reached as a result of their observations in this connection: (1) The total calcium of plasma or serum is nearly all accountable for as protein-bound calcium and calcium ion, the concentration of the latter at any one time being the resultant of an equilibrium between the total calcium and the total protein present in the plasma. (2) The calcium ion concentration of the plasma is normally maintained within a relatively narrow range, 4.25 to 5.25 mg. per 100 cc., by a process of physiologic regulation in which the parathyroid glands play an important role. (3) Practically all of the calcium of protein-free body fluids, such as cerebrospinal fluid, is in ionized form. (4) The calcium ion concentration may be calculated in human body fluids by means of a mass law equation, expressing the relationship between calcium and protein, with a clinically satisfactory degree of accuracy. A chart has been constructed, by means of which the concentration of ionized and protein-bound calcium may be readily derived from values for total calcium and total protein. According to this hypothesis, therefore, the diffusible fraction of serum calcium is practically completely ionized.

These observations are obviously of great theoretical and practical significance, but there appear to be certain obstacles

in the path of the complete acceptance of this concept of the state of calcium in the blood and other body fluids. As stated by McLean and Hastings, it involves the concept of continuous supersaturation of these fluids with respect to calcium carbonate and calcium phosphate, to which there are many objections. Moreover, as pointed out by Thomson and Collip, the isolated frog heart method, employed for the determination of calcium ion concentration, has yielded the unexpected (and theoretically suspect) result that the addition of phosphate or carbonate to blood serum has little or no effect upon the amount of ionized calcium. Final decision in this important matter must await the results of further investigation of the reliability of this method.

FACTORS INVOLVED IN THE MAINTENANCE OF THE NORMAL SERUM CALCIUM LEVEL AND PARTITION

Parathyroid Hormone. The outstanding physiologic effect of the administration of active extracts of the parathyroid glands is an increase in the calcium concentration of the blood serum. It is not known whether this effect is due to an influence exerted directly upon calcium metabolism or primarily upon phosphorus metabolism, the calcium being affected secondarily. Because of the fact that calcium exists in the body chiefly in the form of calcium phosphate it is obvious that either one of these elements cannot be significantly affected without simultaneously involving some change in the other. Whichever view may be correct, the effect upon the level of blood calcium is unquestionable, and, as stated by Collip, it is believed that the physiologic action of the parathyroid hormone is, normally, to regulate calcium metabolism and to maintain a definite level of calcium in the circulating blood. The production of this characteristic effect is dependent to a certain extent upon the presence of an adequate supply of vitamin D, in the absence of which the injection of the parathyroid hormone may fail to produce an increase in the serum calcium concentration.

The influence of the parathyroid glands upon the partition of serum calcium into its diffusible and nondiffusible components is less definitely established. Some believe that the effect of parathyroid hormone is exerted entirely upon the diffusible calcium whereas others maintain that the nondiffusible fraction is the only one affected. There is evidence that parathyroid extract increases both the diffusible and the nondiffusible portions of the serum calcium. It appears certain that the level of diffusible calcium is not entirely dependent upon the

total serum calcium level and that the diffusible and non-diffusible fractions may vary independently of one another, under abnormal conditions. According to McLean and Hastings one of the important functions of the parathyroid hormone is to maintain the normal calcium ion concentration, which is increased by parathyroid administration and is decreased in hypoparathyroidism. Studies of the diffusibility of calcium into the peritoneum suggest that parathyroid hypercalcemia is due to a roughly proportional increase in both diffusible and non-diffusible fractions in the presence of adequate amounts of mobilizable calcium in the body; the primary fundamental effect of this hormone appears to be, however, to increase the diffusible fraction.^{10,11}

Our knowledge of the mechanism of action of the parathyroid hormone is definitely limited. Its physiologic effects have been briefly outlined by Aub as follows: (1) to raise the blood calcium and lower blood phosphorus; (2) possibly to increase the ionized calcium in the blood; (3) to increase calcium and phosphorus elimination in the urine; (4) to obtain the calcium for this increased demand either from a large amount of ingested calcium or from the stores in the bones. To these may be added the following: (5) to increase serum phosphatase activity; (6) to decrease corpuscular ester phosphorus. The mechanism whereby these effects are produced is still the subject of intensive investigation and considerable controversy.

The most important of the current theories of parathyroid hormone action may be outlined as follows:

(1) Albright and Ellsworth and their collaborators believe the initial effect to be an increased renal excretion of phosphorus, in consequence of which the serum inorganic phosphorus decreases, resulting in a state of relative unsaturation of the serum with regard to calcium phosphate. This leads to mobilization of phosphate, and simultaneously calcium, from the bones, resulting in hypercalcemia and continued increased elimination of phosphorus and calcium in the urine. However, it has been reported recently that hypercalcemia develops following the administration of parathyroid hormone to nephrectomized animals, in whom this mechanism could not have been operative.^{32a} Moreover, characteristic bone lesions of hyperparathyroidism have been produced in nephrectomized rats by administration of parathyroid hormone.^{14,24,32a} These observations appear to eliminate the kidneys as the primary locus of action of the hormone and urinary excretion of phosphorus as essential for the production of the metabolic consequences of parathyroid hormone administration.

(2) Greenwald and Gross advanced the hypothesis that an organic substance secreted by the parathyroid glands, probably identical with the parathyroid hormone or formed under its influence, unites with the ionized calcium of the blood plasma to form an undissociated compound. The concentration of calcium ions in the plasma being thus diminished, calcium is liberated from the bones, with the consequent development of hypercalcemia.

(3) Jaffe believes that the administration of parathyroid hormone results first in the withdrawal of large amounts of calcium and phosphorus from the soft tissues and their subsequent elimination, their mobilization from the bones occurring as a secondary phenomenon. It is suggested that this decalcification results from a modification of the tissue fluids circulating about the bone which favors removal of minerals from the bone rather than their deposition.

(4) Thomson and Collip believe that the hormone acts primarily upon the bones, causing liberation of calcium by a direct, stimulating action, rather than by increasing the solvent power of the blood plasma for calcium compounds. Histologic studies support the view that the parathyroid hormone acts directly upon the bones, stimulating osteoclasts and the formation of increased numbers of osteoclasts. It has been found that the return of the previously increased calcium excretion and increased serum calcium to normal levels in the later stages of hyperparathyroidism coincides with the disappearance of osteoclasts from the bone marrow. Jaffe, on the other hand, believes that the appearance of osteoclasts in large numbers is not a primary or specific hormone effect; he states that osteoclasts phagocytose the bone matrix only after it has been decalcified and that a similar reaction can be produced by procedures that do not involve apparently any stimulation of parathyroid secretion.

Vitamin D and Ultraviolet Irradiation. Both of these agents cause an increase in the concentration of calcium in the blood. It is believed that this effect is produced partly, perhaps, by an increase in the efficiency of absorption of calcium from the intestine and partly by the influence of these agents upon the intermediary metabolism of phosphorus and calcium. Under normal conditions the effect of vitamin D appears to be dependent upon a normal supply of parathyroid hormone, although in the absence of the latter factor a characteristic effect may be produced by the administration of excessively large doses and proper dietary regulation. The effect of vitamin D upon serum

calcium, although identical with that of parathyroid hormone, is probably produced in an entirely different manner.

Some observers believe that the hypercalcemic effect of vitamin D is produced through stimulation of the parathyroid mechanism. This belief is based chiefly upon the similarity of many of the chemical and morphological changes that accompany the administration of these agents in large doses, and the observation of Taylor and others that whereas tetany following parathyroidectomy in dogs might be relieved by vitamin D in large doses, tetany was extremely refractory to such treatment in animals in which all recognizable parathyroid tissue had been removed. However, the following observations may serve to emphasize certain fundamental differences in the physiologic effects of these two agents, which render this hypothesis untenable, in our opinion: (1) It is generally recognized that whereas vitamin D produces a prompt and relatively marked rise in the inorganic phosphorus of the blood plasma, the administration of parathyroid hormone, unless continued to the point of renal functional impairment, causes a relatively slight, more gradual increase, if any, in this element, frequently preceded by a slight primary decrease. (2) Whereas the relief of tetany by vitamin D in parathyroidectomized dogs has been attributed to stimulation of accessory parathyroid tissue, the same response is seen in parathyroidectomized rats, in which parathyroid rests are probably rare. (3) The characteristic histologic picture of osteitis fibrosa, which has been observed in experimental hyperparathyroidism, has not been seen in experimental hypervitaminosis D. (4) The parathyroid hormone does not produce healing of the metaphyseal lesion of rickets and may, in fact, retard healing, although it frequently relieves the associated tetany. (5) It has been shown that vitamin D may be effective in animals and patients immune or highly resistant to parathyroid hormone. Toxic manifestations of hypervitaminosis D have been produced in chickens which were unaffected by repeated large doses of parathyroid hormone. (6) Normal and parathyroidectomized animals show little difference in susceptibility to toxic doses of vitamin D. (7) The serum phosphatase activity is increased in hyperparathyroidism and vitamin D deficiency, the administration of parathyroid hormone causing an increase and of vitamin D in rickets a decrease in serum phosphatase activity. Moreover, it has been found that the administration of parathyroid hormone is followed by a decrease in corpuscular ester phosphorus while the administration of large doses of vitamin D is followed by an increase.^{8,9} (8) Phosphate clearance studies have also revealed an important differ-

ence in the effects of vitamin D and parathyroid hormone. Administration of vitamin D to dogs was found to result in a marked increase in the maximum rate of reabsorption of phosphate by the renal tubules, increasing the concentration of inorganic phosphate in the plasma.²² Administration of parathyroid hormone was followed by a considerable decrease in the rate of reabsorption of phosphate by the renal tubules and a consequent reduction in the plasma phosphate concentration.²² These observations indicate, in our opinion, that the modes of action of these two agents are not fundamentally identical.^{8,9}

Plasma Proteins. Because of the fact that about half of the total serum calcium is bound in some way to the plasma proteins (nondiffusible calcium), alterations in the plasma protein concentration may be expected to result in a similar change in the concentration of calcium. This has been found to be the case, both clinically and experimentally. The albumin fraction is of much greater importance than globulin or fibrinogen in this calcium-binding action. According to Peters and Eiserson, the quantitative relationship between the plasma protein concentration and the serum calcium level, other factors, particularly serum phosphate, being normal, may be expressed by the following equation: Serum Calcium = $0.556 \text{ protein} + 6$.

Greenwald proposed the following formula to express this relationship: Serum Calcium = $x + 0.875 \text{ protein}$, in which x is a quantity which varies between 5.0 and 3.7 in different cases, and is about 6.3 in the blood of infants. The value of x must be determined for each analytical series. As stated by Schmidt and Greenberg, any number of such equations can be derived, all of which may give a fairly good representation of any single series of results, but a completely balanced equation cannot be derived because of lack of sufficient background of underlying knowledge. One obvious difficulty lies in the fact that since the calcium-binding action is restricted largely to the albumin fraction, the total protein concentration of the serum may yield misleading results in such an equation. Moreover, there is evidence that the plasma protein may not be completely "saturated" with calcium under normal conditions and that under such circumstances as following the injection of calcium salts or parathyroid hormone the same quantity of protein may be able to combine with an added amount of calcium.^{9,22}

Serum Phosphate. There seems to be little doubt that, other things being equal, there is a roughly reciprocal relationship between the concentrations of serum calcium and serum inorganic phosphate. Thus, the increased serum phosphate which occurs in renal failure and after the intravenous injection of

phosphate is accompanied by a decrease in serum calcium concentration; the hypocalcemia of hypoparathyroidism is accompanied by hyperphosphatemia while the hypercalcemia of hyperparathyroidism is accompanied by hypophosphatemia, at least in the early stages, before the development of renal functional impairment. Albright suggested that this inverse relationship between calcium and phosphorus in the serum is dependent upon the saturation level of some calcium-phosphate compound, the exact nature of which is not known. However, there are several theoretical objections to this concept. There have been several attempts to express this reciprocal relationship mathematically. Peters and Eiserson have proposed the following formula as a quantitative expression of the relationship between calcium, phosphate and protein: $\text{Serum Calcium} = 0.556 \text{ Protein} + 7 - 0.255 \text{ P}$, in which calcium and phosphate are expressed in milligrams and protein in grams per 100 cc. of serum. However, as was noted above, such formulae should not be relied upon and serve merely to express in a general way the nature of the influence of protein and phosphate upon the serum calcium concentration. Obviously, the diffusible fraction of serum calcium is the portion affected by changes in serum phosphate concentration. According to the concept of Freudenberg and György and of Kugelmass and Shohl, as indicated in the formulae presented above, these changes have a definite effect upon the concentration of calcium ion. According to this hypothesis, as the phosphate ion concentration increases the concentration of calcium ion decreases, and vice versa.

Acid-base Equilibrium. The condition of the acid-base balance may be an important factor in determining the degree of ionization of serum calcium. The formulae referred to above indicate that the concentration of calcium ions varies directly with the hydrogen ion concentration. From a practical standpoint this hypothesis implies that this alteration in calcium ionization, which may frequently be associated with manifestations suggestive of profound disturbance of calcium metabolism, may be unaccompanied by any significant deviation from the normal level of serum calcium. In some cases the intravenous administration of sodium bicarbonate, with the consequent production of alkalosis, has been found to result in diminution in the serum calcium concentration, whereas acidosis produced by ammonium chloride has been found to cause an increase in the serum calcium level. These observations, however, are not in accord with the findings of most investigators.

Miscellaneous. Ingestion of adequate amounts of soluble calcium salts in the postabsorptive state results in elevation of

serum calcium, which reaches a maximum in two to three hours and returns to the previous level in about four hours. After intravenous injection, the peak is reached in a few minutes, with a subsequent fall to normal, usually within one to two hours, depending upon the quantity given. Following intramuscular injection (calcium glucogalactogluconate) a maximum level is reached in about one hour, with a subsequent fall over a period of three to four hours. Intravenous or intramuscular injection of magnesium salts may cause a fall in serum calcium, at times to tetanic levels.²³

CALCIUM CONTENT OF OTHER BODY FLUIDS

It is probable that the cerebrospinal fluid and normal tissue fluids contain the diffusible fraction of serum calcium, namely, 4.5-6 mg. per 100 cc. In the presence of protein in tissue fluids their calcium content is increased by an amount proportional to the quantity of protein present, the added calcium being nondiffusible in nature and the diffusible fraction remaining within the limits above mentioned.

ABNORMAL SERUM CALCIUM

Deviation from the normal state of calcium in the blood serum may be manifested in one of two ways, namely, by an alteration in the total serum calcium concentration or by an alteration in the distribution or partition of calcium in the blood and tissue fluids.

HYPERCALCEMIA^{24 25 26 27}

Hyperparathyroidism. One of the most striking results of the injection of parathyroid hormone is an increase in the level of total serum calcium, the degree of rise being dependent upon the dosage and the frequency of its administration. This hypercalcemia occurs even though calcium is withheld from the diet, the increased quantity of circulating calcium being derived from the bones. One of the most interesting developments of experimental investigation of the action of the parathyroid hormone has been the recognition of a well defined clinical state of hyperparathyroidism associated with and forming the etiologic basis of certain cases of the generalized form of osteitis fibrosa described by von Recklinghausen. Many such cases have been reported in which striking improvement has followed the removal of hyperfunctioning, hyperplastic or adenomatous parathyroid glands. Serum calcium values ranging from 12 to 29.5 mg. per 100 cc. have been observed in these cases, but values above 20 mg. are rare. Occasionally the serum calcium may be

within normal limits, but repeated determinations usually reveal hypercalcemia at some time during the course of the disease. Hypercalcemia is probably invariably present during periods of progression of the skeletal lesions and in the presence of active symptoms. It is probable that there may be periods of temporary latency of the condition, as in the case of hyperthyroidism. There is also experimental basis for the possibility that the serum calcium may return to normal or even subnormal levels following a period of hypercalcemia, with exhaustion of the mobilizable calcium reserves in the bones, particularly if the calcium intake is inadequate. Demonstration of abnormally high calcium and phosphate excretion in the urine (p. 185) is of great diagnostic importance under such circumstances as well as in all questionable cases.

In uncomplicated cases, the increase in serum calcium is accompanied and, in fact, preceded by a decrease in serum phosphate, the latter phenomenon being of considerable diagnostic significance (p. 192). Occasionally, in cases of primary hyperparathyroidism complicated by renal failure, a not uncommon complication (nephrocalcinosis, nephrolithiasis, pyelonephritis), the serum calcium concentration may fall to normal or even subnormal levels coincidently with an increase in the serum phosphate concentration (pp. 182, 394).

The mechanism underlying the production of hypercalcemia in this condition has been considered in the discussion of the action of the parathyroid hormone. It appears that both the diffusible and nondiffusible fractions participate approximately equally in the serum calcium increase, although there is some question as to whether or not the primary increase occurs only in the diffusible fraction.⁸ The hypercalcemia of induced hyperparathyroidism is accompanied by the following phenomena:^{8,10} (1) primary slight decrease in serum phosphate, followed later by progressive increase, due probably to renal functional impairment; (2) diminution in corpuscular ester phosphorus; (3) increased serum phosphatase activity; (4) increased urinary calcium and phosphorus elimination, most marked on a low calcium and phosphorus intake; (5) hemoconcentration, with an increase in serum protein concentration; (6) hypochloremia, due probably to excessive loss of chloride by diuresis.

The existence of a state of secondary hyperparathyroidism has been assumed in other conditions in which parathyroid hyperplasia may develop. These include:

(a) Chronic glomerulonephritis and other forms of renal disease accompanied by "renal rickets."

(b) True rickets and osteomalacia.

(c) Certain cases of senile osteoporosis, multiple myeloma, pituitary basophilism (Cushing's syndrome), metastatic malignancy involving the skeleton, acromegaly, osteogenesis imperfecta, "marble bones" disease and chronic hypertrophic arthritis.

(d) Pregnancy.

In some of these, *e.g.*, "renal rickets," rickets and osteomalacia, some observers have reported an increase in parathyroid hormone in the blood, as determined by the rabbit hypercalcemia test of Hamilton and Schwartz (p. 187). However, the validity of interpretations based upon results obtained by this procedure is open to question.

Hypervitaminosis (Vitamin 'D). Hypercalcemia may result from the administration of excessive quantities of the antirachitic factor (vitamin D). Clinically, this condition probably rarely occurs, for, experimentally, extremely large doses must be administered before definitely toxic effects are produced. However, there appears to be a wide range of individual susceptibility to the introduction of this factor and the coincident administration of large quantities of calcium salts increases the tendency toward the production of hypercalcemia. The administration of therapeutic doses of dihydrotachysterol (A. T. 10), a substance chemically related to vitamin D, results in an increase in serum calcium. The physiologic effect of this substance appears to be more closely analogous to that of the parathyroid hormone than does that of vitamin D.

Nephritis. The serum calcium may be increased in rare cases of advanced nephritis with uremia. This is possibly brought about through the combined effects of defective elimination and increased hydrogen ion concentration. Occurring rarely in this condition, it is difficult to explain on any well-founded basis. Some evidence has been presented in support of the view that chronic nephritis is accompanied by a state of hyperparathyroidism, which may possibly account for the occasional observation of hypercalcemia in this condition. However, the majority of observers believe that the enlargement of the parathyroid glands which occurs commonly in nephritis is compensatory in nature and that a state of true hyperfunction of these glands probably is not present.

Polycythemia Vera. Hypercalcemia has been observed occasionally in patients with polycythemia vera, the serum calcium concentration in one reported series ranging from 11.1 to 18.1 mg. per 100 cc., the average figure being 14.3 mg. The cause for this hypercalcemia is not known. A reduction in the red cell count was associated with diminution in the level of serum

within normal limits, but repeated determinations usually reveal hypercalcemia at some time during the course of the disease. Hypercalcemia is probably invariably present during periods of progression of the skeletal lesions and in the presence of active symptoms. It is probable that there may be periods of temporary latency of the condition, as in the case of hyperthyroidism. There is also experimental basis for the possibility that the serum calcium may return to normal or even subnormal levels following a period of hypercalcemia, with exhaustion of the mobilizable calcium reserves in the bones, particularly if the calcium intake is inadequate. Demonstration of abnormally high calcium and phosphate excretion in the urine (p. 185) is of great diagnostic importance under such circumstances as well as in all questionable cases.

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calcium. In most cases of this condition the serum calcium has been found to be within normal limits.

Multiple Myeloma. Serum calcium values ranging from normal to 20.2 mg. per 100 cc. have been observed in patients with multiple myeloma. Hypercalcemia has been reported in about 50 per cent of the cases in which mention was made of the serum calcium concentration.¹⁹ Some have attributed this phenomenon to the existence of a state of hyperparathyroidism. However, this hypothesis is contradicted by the usual finding in multiple myeloma of a normal or elevated serum phosphorus concentration (renal functional impairment) and usually normal serum phosphatase activity. The increase in serum calcium may be dependent in some cases upon the frequently increased serum protein concentration. However, the fact that the increase in serum protein, when it occurs, is practically always confined to the globulin fraction, renders this possibility unlikely. No definite statement can be made at the present time regarding the pathogenesis of hypercalcemia in multiple myeloma.

Increased CO₂ Tension. Hypercalcemia of mild degree (11.5-12.7 mg. per 100 cc.) is occasionally found in conditions in which the CO₂ tension of the blood is increased, thereby enhancing its capacity for maintaining calcium in solution. It has been observed in patients with chronic emphysema, pneumonia, pneumoconiosis and pulmonary congestion secondary to myocardial insufficiency. It may also occur in asphyxia due to any cause.

Neoplastic Disease of Bone. The serum calcium concentration is usually within normal limits in the great majority of cases of primary and metastatic neoplasms of bone. However, values as high as 22 mg. per 100 cc. have been reported, particularly in cases of extensive metastatic involvement of the skeleton. In such cases, values for serum phosphorus and serum phosphatase activity are usually within normal limits, except in the case of osteogenic sarcoma, in which the serum phosphatase activity may be increased.

Ovulation in Birds. Marked hypercalcemia has been demonstrated in birds just before and during ovulation. Serum calcium values ranging from 20 to 28 mg. per 100 cc. have been reported during this period in chickens. It has been shown that these changes are not correlated with the high calcium requirement for shell formation. A similar phenomenon has been observed following artificial distention of the oviduct with paraffin and in one instance in a nonlaying hen in which the oviduct was found to be distended with fluid. It has been suggested that the hypercalcemia accompanying both ovulation and artificial

distention of the oviducts may be dependent upon indirect stimulation of parathyroid secretion as a result of increased pituitary activity (parathyrotrophic hormone of the anterior hypophysis). However, the existence of a parathyrotrophic hormone has not been conclusively established.

Acute Bone Atrophy. Hypercalcemia, with increased urinary excretion of calcium and phosphorus, may occur as a result of rapid demineralization of bones, especially in the presence of simultaneously impaired renal function. This has been observed particularly in children, during periods of immobilization of the extremities due to fracture or paralysis (poliomyelitis). In contrast to hyperparathyroidism, the serum phosphate is either normal or elevated.

Miscellaneous. Administration of anterior hypophyseal extracts to animals has resulted in hypercalcemia. However, except for the possible hypophyseal origin of hyperparathyroidism due to diffuse hyperplasia or hypertrophy of the parathyroid glands, significant hypercalcemia has not been observed in uncomplicated clinical pituitary disorders. Borderline high values occur occasionally in patients with pituitary basophilism. A similar effect has been observed following administration of gonadotrophic hormone and estrogens to certain experimental animals.

HYPOCALCEMIA

Hypoparathyroidism. Hypocalcemia is one of the most constant features of diminished parathyroid function. Until comparatively recently it has been assumed to be due entirely to a decrease in diffusible calcium, but recent work appears to indicate that this may not always be the case. As a rule, when the serum calcium falls below 7 mg. per 100 cc. due to parathyroid deficiency, symptoms of tetany are manifest. In typical cases the serum phosphate concentration is increased.

Clinically, this condition occurs in two forms, (a) postoperative, following thyroidectomy or parathyroidectomy and (b) idiopathic, a condition analogous to spontaneous hypothyroidism. A third rare variety may follow hemorrhage or inflammation in the deep cervical tissues or in the glands themselves. Postoperative tetany may result from accidental removal of the parathyroid glands during thyroidectomy or from excision of too much parathyroid tissue in parathyroidectomy for hyperparathyroidism. Occurring after thyroidectomy, it is usually due to temporary suppression of parathyroid function as a result of trauma, edema or hemorrhage or interference with the blood supply.

calcium. In most cases of this condition the serum calcium has been found to be within normal limits.

Multiple Myeloma. Serum calcium values ranging from normal to 20.2 mg. per 100 cc. have been observed in patients with multiple myeloma. Hypercalcemia has been reported in about 50 per cent of the cases in which mention was made of the serum calcium concentration.¹⁹ Some have attributed this phenomenon to the existence of a state of hyperparathyroidism. However, this hypothesis is contradicted by the usual finding in multiple myeloma of a normal or elevated serum phosphorus concentration (renal functional impairment) and usually normal serum phosphatase activity. The increase in serum calcium may be dependent in some cases upon the frequently increased serum protein concentration. However, the fact that the increase in serum protein, when it occurs, is practically always confined to the globulin fraction, renders this possibility unlikely. No definite statement can be made at the present time regarding the pathogenesis of hypercalcemia in multiple myeloma.

Increased CO₂ Tension. Hypercalcemia of mild degree (11.5-12.7 mg. per 100 cc.) is occasionally found in conditions in which the CO₂ tension of the blood is increased, thereby enhancing its capacity for maintaining calcium in solution. It has been observed in patients with chronic emphysema, pneumonia, pneumoconiosis and pulmonary congestion secondary to myocardial insufficiency. It may also occur in asphyxia due to any cause.

Neoplastic Disease of Bone. The serum calcium concentration is usually within normal limits in the great majority of cases of primary and metastatic neoplasms of bone. However, values as high as 22 mg. per 100 cc. have been reported, particularly in cases of extensive metastatic involvement of the skeleton. In such cases, values for serum phosphorus and serum phosphatase activity are usually within normal limits, except in the case of osteogenic sarcoma, in which the serum phosphatase activity may be increased.

Ovulation in Birds. Marked hypercalcemia has been demonstrated in birds just before and during ovulation. Serum calcium values ranging from 20 to 28 mg. per 100 cc. have been reported during this period in chickens. It has been shown that these changes are not correlated with the high calcium requirement for shell formation. A similar phenomenon has been observed following artificial distention of the oviduct with paraffin and in one instance in a nonlaying hen in which the oviduct was found to be distended with fluid. It has been suggested that the hypercalcemia accompanying both ovulation and artificial

Milk Fever of Cattle. Recent studies have demonstrated that hypocalcemia is not only constantly present but also appears to be of fundamental importance in the etiology of this condition. A reduction of 50 per cent or more in the serum calcium concentration has been observed in many cases.

TABLE 4

CONDITIONS COMMONLY ASSOCIATED WITH ALTERATION IN SERUM CALCIUM CONCENTRATION AND PARTITION

Condition	Total Ca, mg. per 100 cc	Diffusible Ca, mg per 100 cc	Nondiffusible Ca, mg. per 100 cc.	Phosphate, mg per 100 cc	Protein, Gm per 100 cc	pH
Normal . . .	8.5-11.5	4.5-6	4-5.5	3-4.5	6-8	7.3-7.5
Tetany:						
Parathyroid	Decrease	Decrease	Normal or decrease	Increase	Normal	Normal
Postoperative	Decrease	Decrease	Normal or decrease	Increase	Normal	Normal
Maternal	Decrease	Decrease	Normal or decrease	Normal or increase	Normal or decrease	Normal
Infantile	Decrease	Decrease	Normal	Decrease or normal	Normal	Normal
Osteomalacic	Decrease	Decrease	Normal	Normal or decrease	Normal	Normal
Gastric . .	Normal	Normal	Normal	Normal	Normal	Increase
Hyperventilation	Normal	Normal	Normal	Normal	Normal	Increase
Bicarbonate	Normal	Normal	Normal	Normal	Normal	Increase
Sprue	Decrease	Decrease	Normal	Normal	Normal	Normal
Celiac disease	Decrease	Decrease	Normal	Normal	Normal	Normal
Pregnancy	Decrease	Increase	Decrease	Normal	Normal or decrease	Normal or decrease
Hyperparathyroidism	Increase	Increase	Normal or increase	Decrease	Normal	Normal
Kala-azar	Decrease	Normal	Decrease	Normal	Decrease	Normal
Nephrosis	Decrease	Normal	Decrease	Normal	Decrease	Normal

Maternal Tetany. The physiologic changes in calcium and phosphorus metabolism in pregnancy are discussed elsewhere (pp. 518, 519). The requirement for these elements and for vitamin D is increased and the occurrence of parathyroid hyperplasia indicates the increased functional demands upon these structures. Under normal conditions the serum calcium tends to fall, but not to subnormal levels, during the late months of pregnancy and during lactation, but it may become subnormal occasionally, with mild manifestations of tetany. The etiology of this condition is not clear, but it appears to be dependent in some cases upon deficiency in calcium and vitamin D and in others upon parathyroid deficiency operating alone or in conjunction with other factors. The interesting condition of "tetany of the newborn" appears to be related to the existence of tetany in the mother. It is due perhaps to inability of the maternal parathyroid glands to compensate fully for the fetus and to the fact that the fetal parathyroids are not fully functioning at birth in such cases.

Vitamin D Deficiency (Rickets and Osteomalacia). Deficiency in vitamin D (p. 326) results characteristically in rickets in young children and osteomalacia in older children and adults. In the majority of cases the serum calcium is within normal limits, the serum phosphate being characteristically decreased and the serum alkaline phosphatase activity increased. In some instances, however, especially when the calcium intake is low, hypocalcemia occurs (4-7.5 mg. per 100 cc.), with manifestations of tetany (infantile tetany; spasmophilia; osteomalacic tetany).

Steatorrhea (Sprue, Cellac Disease). The outstanding feature of these disorders is defective absorption of fat from the intestine, as a result of which large quantities of calcium soaps are formed, which are poorly soluble and consequently poorly absorbed. There is probably also deficient absorption of vitamin D. Impaired absorption in these conditions is contributed to by the increased intestinal motility. These abnormalities lead to osteoporosis, rickets, dwarfism, osteomalacia, hypocalcemia and tetany. The serum phosphate is often low and excessive amounts of calcium and phosphate are lost in the feces.

Hunger Osteopathy. This condition appears to be due to a diet deficient in total calories, calcium, phosphorus and vitamin D, and resembles a slowly progressive osteomalacia.

Nephrosis. Serum calcium values ranging from 5.7 to 9.1 mg. per 100 cc. have been observed in patients with nephrosis and with chronic glomerulonephritis with a nephrotic complement. This diminution in serum calcium is due entirely to a decrease in the nondiffusible fraction which occurs as a result of a marked diminution in the concentration of serum protein, which is one of the most characteristic features of this condition. In no case is there any significant alteration in the amount of diffusible calcium and increased neuromuscular excitability is not observed.

Nephritis. Hypocalcemia is occasionally observed in chronic glomerulonephritis and nitrogen retention. It occurs usually in the later stages of this condition and is associated with and perhaps dependent upon the increase in serum phosphate which occurs in these cases. In advanced nephritis with uremia, inorganic phosphorus figures of 12-20 mg. per 100 cc. may be found to be associated with serum calcium values of 4-6 mg. per 100 cc. Tetany may occur as a result of such marked lowering of the serum calcium concentration. As was indicated above, the concentration of calcium in the serum varies roughly inversely with that of phosphorus.

Kala-azar. Hypocalcemia in this condition, as in nephrosis, is dependent upon a decrease in the concentration of serum protein.

ABNORMAL URINE CALCIUM

INCREASED URINARY CALCIUM

Hyperparathyroidism: The injection of parathyroid extract is followed by a gradual increase in the excretion of calcium, this increase taking place entirely in the urinary calcium, the fecal excretion being unaffected. Although in most cases the increased elimination is dependent upon a rise in the level of serum calcium, it may occur in the absence of any such elevation. Similar observations have been made in clinical states of hyperparathyroidism (*osteitis fibrosa diffusa*). Whereas under normal conditions 70-90 per cent of the excreted calcium is eliminated in the feces and 10-30 per cent in the urine, in hyperparathyroidism these proportions are reversed, 70-90 per cent being eliminated in the urine and only 10-30 per cent in the feces. Patients suffering with this condition cannot be maintained in calcium equilibrium upon a daily intake sufficient for normal individuals (0.45 Gm. calcium daily). This negative balance is best demonstrated when the intake of calcium and phosphorus is maintained at a low level for test periods of at least three days (calcium 0.11 Gm. and phosphorus 0.4 Gm. daily). This test procedure is of diagnostic value in early or mild cases of hyperparathyroidism, when the abnormalities in serum calcium and phosphorus are not striking or characteristic.

Hyperthyroidism. Increased thyroid secretion is associated with a marked accentuation of calcium excretion, the urinary calcium being increased somewhat out of proportion to the fecal calcium and averaging about 56 per cent of the total output. This appears to be due to a direct stimulating catabolic effect on the calcium deposits in the bones similar to the general action of thyroid extract upon other body tissues.

Acidosis. Acidosis, whether due to the ingestion of mineral acids or acid-forming substances or associated with disease states, is accompanied by an increase in calcium excretion, the excess calcium, as in the case of hyperthyroidism and hyperparathyroidism, being in all probability abstracted from the bones. This increased excretion of calcium is due almost entirely to an increase in urinary calcium.

Hypervitaminosis D. The administration of extremely large quantities of vitamin D or of smaller doses of dihydrotachysterol (A. T. 10) may result in increased excretion of calcium in the urine similar to that observed in hyperparathyroidism. In the case of vitamin D, this may represent a toxic rather than a physiologic effect.

Magnesium, Phosphate, Oxalate and Citrate Tetany. Intravenous or intramuscular injection of magnesium salts may cause a fall in serum calcium, at times to tetanic levels.²³

A fall in serum calcium, with signs of tetany, may follow intravenous injection of neutral, alkaline or slightly acid sodium phosphate. That tetany does not occur after injection of acid sodium phosphate, even though the serum calcium falls, is due probably to increase in the ionization of calcium incident to the accompanying acidosis. The hypocalcemia of chronic renal failure probably results chiefly from phosphate retention (p. 182).

Parenteral administration of soluble oxalates and of citrates produces tetany, the former by the production of hypocalcemia through the formation of insoluble calcium oxalate, the latter by formation of a poorly ionized calcium citrate compound, without hypocalcemia.

Miscellaneous. Slight decrease in serum calcium concentration has been reported occasionally in patients with prolonged obstructive jaundice, malignancy and other cachectic conditions. It would appear that hypocalcemia under such circumstances is dependent primarily upon a decrease in plasma albumin concentration and is not due to primary disturbance in calcium metabolism.²⁹ The decrease occurs entirely in the nondiffusible fraction of the serum calcium and is not accompanied by manifestations of tetany.

ALTERATION IN DISTRIBUTION OF CALCIUM

Pregnancy. During the course of normal pregnancy and early labor there is a gradual diminution in the level of total serum calcium which, however, rarely falls below the low limit of normal. There also appears to be an alteration in the distribution of calcium as evidenced by a progressive decrease in the nondiffusible fraction and a slight increase in the diffusible (cerebrospinal fluid calcium) fraction.

Alkalosis. It is probable that in alkalosis there is a diminution in the proportion of ionized calcium which is responsible for the symptom complex, tetany, so characteristic of alkalotic states. The serum calcium in these conditions is usually within normal limits, the disturbance probably taking place in accordance with the ionization formula of Kugelmass and Shohl previously referred to. This condition occurs clinically following the administration of excessive quantities of alkali (bicarbonate tetany), in states of hyperventilation (hyperpneic tetany) and in association with pyloric and acute upper intestinal obstruction or excessive vomiting from any cause (gastric tetany), the level of total serum calcium being normal in each instance.

malacia, however, although defective absorption may be of importance, it is believed by many that the fundamental defect is in the intermediary metabolism of calcium and phosphorus, whereby normal ossification cannot occur and an increased quantity of calcium is eliminated into the lower bowel. Conversely, calcium retention may be produced by the administration of excessive quantities of vitamin D with consequent diminution in the amount of calcium eliminated in the feces.

DETECTION OF EXCESSIVE PARATHYROID HORMONE IN THE BLOOD

Hamilton and Schwartz and Hamilton and Highman have proposed a method for the determination of excessive amounts of parathyroid hormone in the blood. The rationale of the test is as follows: If calcium chloride is administered at regular intervals to rabbits by stomach tube, it is found that the rise in serum calcium becomes less and less after each administration. If, however, parathyroid hormone, or any substance containing it in sufficient amount, is given to the animals intramuscularly before the administration of calcium, the rise in serum calcium is quite marked also after the later administrations of calcium chloride. The figure by which the results are judged is the difference between the serum calcium of the rabbit before the experiment and the highest value obtained three or five hours later.

The procedure recommended is as follows: A full-grown rabbit, which has been kept on a well-balanced ration, preferably the Steenbock-Bills stock diet, for at least three to five days, is injected intramuscularly with 30 cc. of the blood of the patient to be investigated. Calcium chloride (0.276 Gm., containing 100 mg. of calcium) is given the rabbit by stomach tube immediately after injection and one, three and five hours later. Blood is drawn from the rabbit (marginal ear vein or cardiac puncture) before beginning the test and seven minutes after each of the last two administrations of calcium chloride. A rise of 1.2 mg. per 100 cc. or more in serum calcium concentration is regarded as indicative of the presence of an abnormally large amount of parathyroid hormone in the injected blood.

It has been found that this rise is not proportional to the amount of parathyroid hormone present and, since different animals react differently to identical quantities of the hormone, accurate quantitative information cannot be obtained by this method. However, the authors claim that it may be used to detect the presence of excessive amounts of this substance.

DECREASED URINARY CALCIUM

Hypoparathyroidism. In hypoparathyroidism, hypocalcemia is associated with a diminution in the urinary excretion of calcium. Recent studies appear to indicate that there is a threshold for urinary calcium excretion since the elimination of calcium in the urine decreases abruptly as soon as the serum calcium falls below 6.5-8.5 mg. per 100 cc.

Vitamin D Deficiency. Urine calcium is decreased in vitamin D deficiency, as occurs in rickets and osteomalacia and perhaps also in certain cases of steatorrhea (sprue, celiac disease). This is perhaps due largely to inadequate absorption of calcium from the intestine.

Hypothyroidism. In myxedema, the excretion of calcium has been found to be approximately 40 per cent below the normal average, the diminution in urinary calcium being somewhat greater proportionately than that in fecal calcium.

Ingestion of Bases. It has been found that the administration of base-forming diets causes a decrease in urinary calcium. It is probable that these effects are due largely to decreased absorption from the intestine rather than to any direct influence upon the intermediary metabolism of calcium.

Miscellaneous. An unusually low excretion of calcium, as compared with normal subjects, has been reported in patients with calcinosis universalis maintained on a low calcium and phosphorus intake. A marked diminution in the output of calcium in the urine is a rather constant finding in chronic nephritis. This is due in part to impaired renal excretion of calcium and in part to inadequate absorption of calcium resulting from excretion into the intestine of large amounts of phosphorus which would have been excreted in the urine under conditions of normal renal function. This, together with the accompanying acidosis, may result in the condition known as "renal dwarfism" or "renal rickets," an occasional accompaniment of chronic renal failure in children. Some believe that secondary hyperparathyroidism plays a part in the pathogenesis of the skeletal changes in this condition.

ABNORMAL FECAL CALCIUM

An increase in the quantity of calcium eliminated in the feces may be observed in rickets and osteomalacia (vitamin D deficiency), in sprue and in celiac disease. In the last two conditions this increase is in all probability due to defective calcium absorption resulting from the formation of large quantities of insoluble calcium soaps in the intestine. In rickets and osteo-

Chapter V

Inorganic Phosphorus Metabolism

ABSORPTION AND ELIMINATION⁴⁻⁷

SIMPLE phosphates (Ca, Na, K) are absorbed as such, largely in the small intestine, and more readily in its upper than its lower portion. In the course of digestion of nucleoproteins and phosphoproteins, phosphate is split off and absorbed as such. If ester forms are present they must undergo hydrolysis by phosphatases prior to absorption. For example, liberation of phosphate from lecithin and the carbohydrate esters of phosphoric acid does not occur until they have been subjected to the action of pancreatic and intestinal secretions. It has been suggested that because of this fact a considerable fraction of the ingested phosphorus is absorbed later than the major portion of the calcium. This permits absorption of portions of both elements that might not otherwise be absorbed, especially in regions of low acidity.

The absorption of phosphates from the intestine is governed by essentially the same factors as those which influence the absorption of calcium. Accordingly, marked diminution in the alkaline reaction in the upper intestine favors phosphate absorption; the presence of a relatively large proportion of calcium or magnesium tends to inhibit phosphate absorption due perhaps to the formation of insoluble, tertiary calcium phosphate. The absorption of phosphate from the alimentary tract is also dependent to a large degree upon the utilization of calcium in the body. Some is undoubtedly absorbed in the upper intestine. A large amount of phosphate, however, is secreted into this portion of the bowel and is reabsorbed in the lower intestine, the amount of reabsorption being inversely proportional to the excretion of calcium into the lower bowel. If there is decreased utilization of calcium in the tissues this element is excreted in relatively large quantity and the absorption of phosphate is correspondingly diminished. Vitamin D deficiency, by diminishing absorption of calcium, may thus result in secondary impairment of absorption of phosphorus.

Inorganic phosphorus is excreted by the kidneys and the bowel, usually somewhat more in the urine than in the feces,

Our experience with this procedure has not been satisfactory. The most important objection appears to be the marked fluctuation which occurs in the serum calcium of normal rabbits under ordinary conditions and the common experience that parathyroid hormone fails to produce regular and consistent elevation in the serum calcium concentration of this species.

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HYPERPHOSPHATEMIA

Hypervitaminosis (Vitamin D). The serum phosphate concentration may be decidedly increased by therapeutic or excessive doses of vitamin D in the form of codliver oil or viosterol. Similar results may be obtained by ultraviolet irradiation.

Hypoparathyroidism. Diminished parathyroid function is associated with a slight rise in the serum phosphate concentration which is more or less proportional to the diminution in serum calcium.

Renal Failure. Increase in the concentration of phosphate in the serum of patients with chronic glomerulonephritis is an indication of renal functional insufficiency.⁴ Phosphate retention in nephritis appears to contribute to the acidosis which occurs in the later stages of that condition, high values for serum phosphate being almost invariably accompanied by a decrease in the alkali reserve. However, acidosis may exist in the absence of demonstrable phosphate retention. Hyperphosphatemia in nephritis has approximately the same clinical significance as creatinine retention, values above 8 mg. per 100 cc. in adults being of serious prognostic import. Values as high as 40 mg. per 100 cc. have been reported. Similar alterations in the serum phosphate concentration may occur in renal insufficiency associated with nephrosclerosis, multiple myeloma and destructive kidney lesions such as congenital polycystic kidney, tuberculosis, malignancy, pyonephrosis, pyelonephritis and hydronephrosis. An increase in serum inorganic phosphorus concentration has also been observed in acute high intestinal obstruction.² This may possibly be dependent upon or associated with the state of renal functional insufficiency which occurs in that condition. A similar change has been observed after injection of histamine.

Healing Fractures. During the period of healing of fractures in adults the serum phosphate concentration is often slightly increased, values ranging from 5-7 mg. per 100 cc. being frequently observed.

HYPOPHOSPHATEMIA

Rickets. In rachitic children, serum phosphate values of 1-2 mg per 100 cc. are commonly observed.⁸ This condition is believed to be due fundamentally to vitamin D deficiency, which operates either by diminishing the degree of absorption of phosphorus and calcium or by preventing their proper utilization in the process of ossification. In some cases of rickets the serum phosphate concentration may be within normal limits, the serum calcium being diminished (low calcium rickets). Under such

the relative proportions varying considerably under different conditions. The source of inorganic phosphorus in the urine is chiefly the blood plasma, although there is some evidence that it may be contributed to by hydrolysis of phosphoric acid esters by phosphatase activity in the kidney. On a balanced diet, the urinary phosphate constitutes about 60 per cent of the total excretion. As the calcium intake is decreased, the proportion of phosphorus eliminated in the urine increases, being about 75 per cent of the total with a low calcium, moderately high phosphorus intake. The "renal threshold" for phosphorus excretion is about 2-3 mg. of P per 100 cc. of plasma, excretion falling to a minimum at lower concentrations.

Vitamin D increases and parathyroid hormone decreases reabsorption of phosphorus by the renal tubular epithelium,² the former thus diminishing and the latter increasing urinary excretion of phosphorus. The urinary acidity is regulated to a large extent by the relative proportions of acid (H_2PO_4) and alkaline (HPO_4) phosphates excreted; this constitutes an important part of the mechanism whereby the kidneys conserve base and regulate the acid-base balance of the body fluids (pp. 275, 346). From a clinical standpoint, interest is centered particularly in the phosphate content of the blood serum and urine.

NORMAL SERUM PHOSPHATE

The inorganic phosphate content of blood serum ranges from 3 to 4.5 mg. per 100 cc. in adults and from 5 to 6.5 mg. per 100 cc. in children. Under normal conditions the blood phosphate level varies directly with the concentration of solar ultra-violet rays, being highest in summer and falling during the winter months. Variations in the phosphate content of the serum occur normally during periods of varying carbohydrate utilization, due to the fact that combinations of carbohydrate and phosphoric acid (hexose-phosphate) play an important part in the intermediary metabolism of carbohydrate (p. 15). Following the ingestion of carbohydrate there is a gradual and progressive fall in serum phosphate which persists during the period of increased glucose utilization, returning to normal in four to five hours. This fall occurs independently of the blood sugar level since it depends entirely upon the utilization of glucose in the tissues. A similar drop follows administration of insulin or epinephrine (p. 15). A slight increase in serum phosphorus follows ingestion of calcium and a considerable drop follows parenteral administration of magnesium salts.

with diabetes, but the action of epinephrine in this connection is dependent upon the presence of an adequate supply of insulin, the hypophosphatemic response being diminished in diabetes. Consistently subnormal serum phosphate concentrations may occasionally be observed in individuals suffering with hyperinsulinism. The injection of pituitrin, which, like that of epinephrine, is followed by an increase in the blood sugar content, differs from the latter, however, in causing an increase in the serum phosphate concentration, this effect being interpreted as due to an insulin inhibitory action favoring the dissimilation of the hexosephosphate compound in the tissues.

ABNORMAL URINARY PHOSPHATE

One of the important means by which the kidney aids in maintaining the acid-base balance resides in its ability to transform the basic phosphate (B_2HPO_4) of the blood to the acid phosphate (BH_2PO_4) which is eliminated in the urine. In the presence of acidosis or factors which tend to produce acidosis, the elimination of acid phosphate in the urine is greatly increased, constituting one of the compensatory measures which aid in the maintenance of the acid-base balance. This condition of phosphaturia is not observed in the acidosis of advanced nephritis, which is commonly associated with retention of phosphate as well as other constituents of the blood which are normally eliminated in the urine.

One of the first demonstrable effects of the administration of parathyroid hormone is an increase in the quantity of phosphate eliminated in the urine. This is believed by some observers to be the cause of the subsequently occurring hypophosphatemia and hypercalcemia. Likewise, an increase in urinary phosphate has been observed in clinical hyperparathyroidism (osteitis fibrosa diffusa). In hypoparathyroidism (parathyroid tetany), on the other hand, the urinary phosphate is diminished in quantity. The urinary phosphate is diminished in rickets, the greater part of the ingested phosphate being excreted in the feces, owing perhaps in part to inadequate absorption. The administration of vitamin D or irradiation with ultraviolet light increases the urinary elimination of phosphate in rickets and osteomalacia.

It has been found that both urinary and serum phosphate are decreased following anesthesia produced by ether, chloroform and ethylene. These changes are believed to be secondary to the hyperglycemic effect of these anesthetic agents (see p. 16).

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circumstances the rachitic condition is commonly complicated by tetany (infantile tetany). It was pointed out by Howland and Kramer that if the concentration of calcium be multiplied by that of phosphate, each expressed in milligrams per 100 cc. of blood serum, a product is obtained which, in the normal child, ranges from 50 to 60. When this product is below 30 rickets is invariably present and when it is above 40 either healing is occurring or rickets has not been present.

Osteomalacia. The metabolic disturbance in osteomalacia is believed to be similar to that in rickets, both being probably dependent upon vitamin D deficiency. Diminution in the concentration of serum phosphate is one of the most constant features of this condition.

Idiopathic Steatorrhea. Under this designation are included conditions commonly termed celiac disease, sprue and non-tropical sprue. The characteristic fatty diarrhea in these conditions is frequently accompanied by skeletal demineralization, dwarfism, low serum calcium and phosphorus and characteristic manifestations of rickets and tetany. These abnormalities of calcium and phosphorus metabolism are generally regarded as secondary to the excessively large quantity of fat in the intestine and as due to defective absorption of calcium, phosphorus and probably vitamin D.

Hyperparathyroidism.^{1,6} The injection of parathyroid hormone is followed by a diminution in the concentration of serum phosphate which is perhaps a result of the increased elimination of phosphate in the urine. Similar observations have been made in patients with hyperparathyroidism (osteitis fibrosa diffusa). The decrease in serum phosphate is roughly proportional to the degree of elevation of serum calcium in this condition. Following the repeated administration of large doses of parathyroid hormone, with a maintained serum calcium concentration between 15 and 20 mg. per 100 cc., the elimination of phosphate in the urine diminishes and the serum phosphate begins to rise. This effect upon serum phosphate is due not directly to the action of the parathyroid hormone but to the development of renal functional insufficiency which is also manifested at the same time by an increase in the concentration of the nonprotein nitrogenous constituents of the blood.

Increased Carbohydrate Utilization. As was mentioned above, the serum phosphate concentration diminishes during periods of increased carbohydrate utilization. The administration of insulin or epinephrine produces this effect apparently by favoring the formation of a hexose-phosphate combination. Insulin produces this effect in both normal individuals and those

with diabetes, but the action of epinephrine in this connection is dependent upon the presence of an adequate supply of insulin, the hypophosphatemic response being diminished in diabetes. Consistently subnormal serum phosphate concentrations may occasionally be observed in individuals suffering with hyperinsulinism. The injection of pituitrin, which, like that of epinephrine, is followed by an increase in the blood sugar content, differs from the latter, however, in causing an increase in the serum phosphate concentration, this effect being interpreted as due to an insulin inhibitory action favoring the dissimilation of the hexosephosphate compound in the tissues.

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Chapter VI

Phosphatase Activity

PHOSPHATASE is an enzyme capable of hydrolyzing practically every monophosphoric ester, both aliphatic and aromatic, including a portion of the phosphoric esters of the circulating red blood cells and those present, in small amounts, in blood plasma (Kay). Originally demonstrated in rice and wheat bran, this agent, which hydrolyzes phosphoric esters with the liberation of inorganic phosphate, has been identified in practically all tissues of a variety of animals. It has been demonstrated in the following situations: mucosa of the small intestine, colon and stomach, kidneys (cortex and medulla), ossifying cartilage, bone, periosteum, teeth, spleen, liver, pancreas, lungs, thyroid, thymus, suprarenal glands, testis, cerebrum, cardiac and skeletal muscle, milk, bile, urine, blood plasma, red blood cells and leukocytes.² In the fetus and growing animal the greatest relative quantity of phosphatase is found in the bones and teeth. In the adult animal, the intestinal mucosa contains the greatest amount per unit of weight (wet tissue), being followed in approximate order in this regard by the renal cortex, whole bone, thyroid, spleen, lungs, suprarenal glands and pancreas, with some variation of this order in different species. It has also been found in the bladder, trachea, aorta and retina of different species of animals.

The phosphatase activity of the majority of tissues studied appears to be dependent upon the action of the same enzyme or, at least, enzymes possessing similar properties. The important properties of mammalian phosphatase have been reviewed by Robison and Kay and, since they have little direct clinical application, will not be considered here in detail. It may be stated, however, that the identity of the phosphatases of bone, intestinal mucosa, kidney and blood plasma has been fairly definitely established on the basis of the similarity of certain factors. Among these are the dissociation constant of the enzyme-substrate compound, the ratio of rates of hydrolysis of various esters, activation by magnesium and the optimum pH for hydrolytic activity. It is well established, however, that the phosphatase enzyme present in red blood cells is different from that in other tissues. It has been shown that synthesis as well

as hydrolysis of the phosphoric esters can be accomplished by phosphatase from bone, kidney, intestine, blood plasma and red blood cells under suitable conditions. It has been found that the serum phosphatase, as well as that of most other tissues, is activated by a variety of substances, among which are magnesium, iron, manganese, cobalt, nickel, ascorbic acid and glycine. There is some evidence that this enzyme is inhibited by cholic acid, copper and zinc.

Four types of phosphomonoesterases of biological significance may be differentiated on the basis of their activity in different pH ranges:²² (1) a type (alkaline phosphatase) with optimum activity at about pH 9.3, present in blood plasma or serum, bone, kidney, intestine, mammary gland, spleen, lung, leukocytes, adrenal cortex and seminiferous tubules; (2) a type (acid phosphatase) with optimal activity at pH 6, occurring in mammalian erythrocytes and yeast; (3) a type (acid phosphatase) with optimum activity at pH 5, occurring in prostatic epithelium, spleen, kidney, blood plasma, liver, pancreas and rice bran; (4) a type (acid phosphatase), with optimal activity at about pH 3 to 4, obtained from taka diastase.

From a clinical standpoint, interest is centered particularly upon the phosphatase activity of the blood plasma or serum.

NORMAL SERUM PHOSPHATASE^{2,22}

Unfortunately, much of the data reported in regard to normal and abnormal alkaline phosphatase activity of the blood plasma cannot be compared quantitatively because of differences in the methods employed for its determination. The procedure developed by Kay and by Jenner and Kay are still widely employed, values obtained (normal 0.10-0.21 units per 100 cc.) being much lower than but not consistently proportional to those obtained by the method of Bodansky which, in our opinion, is to be preferred for clinical as well as experimental use. According to the method of Bodansky, a unit of phosphatase activity is defined as "equivalent to the actual or calculated liberation of 1 mg. of phosphorus as the phosphate ion during the first hour of incubation at 37° C. and pH 8.6, with the substrate containing sodium beta-glycerophosphate, hydrolysis not exceeding 10 per cent of the substrate." Employing this procedure, the range of normal values for plasma or serum alkaline phosphatase activity in adults is 1.5 to 4.0 units per 100 cc., that for children being 5 to 14 units. Greene found the normal mean to be 6.3 units, with a standard deviation of 2.2 units. In our experience, values in adults of less than 11 units are of little clinical significance in hospital patients, in the absence of other significant abnormali-

ties. According to Stearns and Warweg, the alkaline phosphatase activity of plasma is low at birth, rises rapidly to a maximum during the first month of life and remains fairly high during the second year, the values in later childhood falling to within the normal adult range.

Bodansky found that the alkaline phosphatase activity of oxalated plasma is about 10 per cent lower than that of serum; this was attributed to dilution of the plasma as a result of the presence of oxalate. Increased activity (about 10 per cent) was found in serum after twenty-four hours' refrigeration and after incubation for four to six hours at 37° C. (16-20 per cent increase). There is some evidence that senility, malnutrition and anemia tend to lower the serum phosphatase activity; this should be taken into consideration in interpreting variations in this factor in clinical conditions. Some observers have found that high protein diets cause a decrease and high carbohydrate diets an increase in alkaline phosphatase activity of the serum, and Bodansky found an increase during alimentary hyperglycemia. It is preferable to speak of phosphatase activity rather than of the quantity of phosphatase enzyme since, as pointed out by Thannhauser, increased values may be dependent upon increased activation of the enzyme by other constituents of the plasma rather than upon an actual increase in the quantity of enzyme.

Normal plasma or serum contains small amounts of acid phosphatase (pH 4.9), less than 3 units per 100 cc.^{10,19} This may have its origin in the liver, spleen, bones, kidney and prostate, but is not entirely or even largely of prostatic origin, since it is present in women and children in essentially the same amounts as in adult men.⁹ This is of interest because of the increase in this factor in prostatic carcinoma (p. 201). Because of the presence of large amounts of an acid phosphatase in the red blood cells, care must be taken to avoid hemolysis in plasma or serum in which this determination is to be made.

ALKALINE PHOSPHATASE IN NORMAL CALCIFICATION AND BONE DISEASE

The hypothesis of Robison and Soames regarding the mechanism of normal calcification of bone may be stated as follows (Kay): "The osteoblasts, the hypertrophic cartilage cells and certain cells of the inner portion of the periosteum in a growing bone contain, or can secrete, a very active phosphatase which, by hydrolyzing the salts of phosphoric esters brought to the ossifying zone by the blood stream, cause a local increase in the concentration of phosphate ions. The solubility product for

calcium phosphate, which is probably very nearly reached at the concentration of inorganic phosphate and ionized calcium normally present in the circulating blood plasma at normal plasma pH, is thus exceeded locally, and a deposition of the calcium phosphate is brought about in, or in the neighborhood of the cells which secrete the active enzyme." Although there is an abundance of acid-soluble esters (phosphoric) in the erythrocytes, these appear to be relatively unavailable as an immediate substrate for the plasma phosphatase and it is probable that the normal substrate is represented by the relatively small quantity of phosphoric ester (averaging about 0.35 mg. per 100 cc.) present in the blood plasma. However, the extremely important question of the normal substrate for plasma phosphatase remains unanswered. The several difficulties of and objections to the phosphatase hypothesis have been thoroughly reviewed by Kay. Suffice it to state here that it seems obvious that this mechanism must be accorded a position of importance in any theory of calcification of bone, not losing sight of the importance of other factors, such as serum calcium and phosphate concentration, the parathyroid hormone, thyroxin, vitamin D, calcium and phosphorus intake and absorption from the bowel and the state of the acid-base balance.

The statement appears justified that an increase in serum phosphatase activity in skeletal disorders is a reflection chiefly of osteoblastic activity. In the light of this fact one may readily understand the lack of consistent relationship between serum phosphatase and the extent of skeletal demineralization in various bone diseases.

Rickets. One difficulty in the path of complete acceptance of the phosphatase hypothesis of calcification of bone is the fact that there is no deficiency in this factor in the zone of hypertrophic cartilage cells in rickets. It has been suggested that the fault may reside in the existing deficiency in plasma inorganic phosphate or in one possible substrate for bone phosphatase, the plasma phosphoric ester content. It is interesting in this connection to note that several observers have reported marked diminution in the phosphoric ester content of the red blood cells in human and experimental rickets and an increase following the administration of large quantities of vitamin D. This observation is of particular significance in the light of the finding of Stearns and Warweg that the ester content of the red corpuscles changes continuously with the growth of the child, rising steadily from birth, reaching a maximum during the second year and then falling gradually until the adult level is reached. It has also been found that the phosphoric ester content of whole blood is

high during the period of most active bone development. These observations suggest that the ester phosphorus of the red blood cells may be the main circulating substrate or store of substrate for the bone enzyme.

The serum phosphatase activity is consistently considerably increased in active rickets. Bodansky and Jaffe made the following observations: Serum phosphatase activity, normally 5-15 units per 100 cc. in children (Bodansky method), was 20-30 units in mild rickets, as high as 60 units in marked rickets and over 60 in very marked rickets. Values as high as 190 units were obtained in the last group. These figures may be regarded as reliable criteria of the severity of the condition at the time of first observation. Following the institution of antirachitic therapy the serum phosphatase activity decreases, usually after an interval of four to twelve days. A high normal figure may be reached within two months and is frequently maintained for some time during the period of active repair. When bone reconstruction is complete the serum phosphatase is within normal limits. It has been found that the plasma phosphatase activity may remain above normal even after healing is apparently complete roentgenographically. There is usually a reciprocal relationship between plasma phosphatase activity and inorganic phosphate concentration in rickets. In the opinion of most observers, determination of plasma phosphatase activity may be regarded as a reliable means of detecting latent and active rickets and may be accepted as an index of improvement in this condition.

Hyperparathyroidism (Generalized Osteitis Fibrosa Cystica). A moderate increase in serum phosphatase activity occurs in clinical and experimental hyperparathyroidism.^{3,7} Values as high as 65 units have been reported, but the values usually range between 20 and 40 units. A gradual fall to normal levels has been observed following parathyroidectomy in such cases, associated with increasing calcification of the affected bones.

Osteitis Deformans (Paget's Disease). Increased serum phosphatase activity has been observed rather consistently in patients with osteitis deformans involving several bones. Higher values have been obtained in this condition than in any other clinical disorder. Bodansky and Jaffe found values of 15-125 units in ten cases of the polyostotic variety and 4.9-23.1 units in thirteen cases with localized involvement of one or two bones. Spontaneous healing by sclerosis was associated with relatively low values for serum phosphatase activity. Age appeared to be a factor, the oldest patients showing the lowest values. The finding of moderately elevated serum phosphatase activity, to-

gether with normal values for serum calcium and inorganic phosphorus, is of distinct diagnostic significance in this condition.

Miscellaneous Bone Disorders. Slightly elevated values for serum phosphatase activity. (5-15 units) have been reported occasionally in patients with generalized osteoporosis, marked hyperthyroidism, osteomalacia, metastatic carcinoma involving bone, osteogenic sarcoma, healing fractures, Gaucher's disease with bone resorption and osteosclerosis fragilis generalisata (marble bones).

Essentially normal findings are obtained in most cases of chronic arthritis, senile osteoporosis, osteomyelitis, bone cysts, tumors not involving bone, achondroplasia, cretinism, calcinosis universalis and multiple myeloma. Very high values are encountered occasionally in cases of carcinoma with extensive and widespread metastases involving the skeleton. Slight increases have been reported also in occasional cases of renal rickets and multiple myeloma.

ALKALINE PHOSPHATASE ACTIVITY IN JAUNDICE, HEPATIC AND BILIARY TRACT DISEASE

Obstructive and Hepatocellular Jaundice (p. 460). Several observers have reported increased serum phosphatase activity in a large proportion of patients with both obstructive and hepatocellular jaundice.^{2,3,4,5,6,11} Values as high as 60 units (Bodansky method) have been observed in such cases. Some believe that phosphatase activity tends to parallel the degree of hyperbilirubinemia in obstructive jaundice more closely than in hepatocellular jaundice, although this finding is by no means constant. Some authors^{8,11} feel that the determination of serum phosphatase activity may be of value in differentiating between these two types of jaundice. They believe that whereas in obstructive jaundice serum phosphatase activity and serum bilirubin concentration increase proportionately until the limit of phosphatase activity is reached (40-60 units), in hepatocellular jaundice the serum phosphatase rarely rises above 12 units in spite of a progressive increase in serum bilirubin.

The experience of the majority of observers is not in complete accord with this conclusion. It would appear that although phosphatase activity values above 20 units are obtained about twice as frequently in patients with obstructive jaundice as in those with hepatocellular jaundice, this factor is of distinctly limited value in differential diagnosis because of a wide overlapping of values in the two groups of cases. The following results were obtained in our laboratories:² obstructive jaundice—less than 10 units (Bodansky), 32 per cent; 10-20 units, 26 per cent;

20-30 units, 35 per cent; more than 30 units, 7 per cent; hepatocellular jaundice—less than 10 units, 55 per cent; 10-20 units, 32 per cent; 20-30 units 13 per cent. Moreover, normal serum phosphatase activity has been reported in infants with congenital atresia of the bile ducts. Data presented by Thannhauser and his associates suggest that the high values obtained in a variety of liver and biliary tract conditions may be due to an increase in the activity of the phosphatase enzyme in the blood rather than to an actual increase in the amount of enzyme. This increased activity may be dependent upon variations in the concentration of factors in the blood which stimulate or inhibit the activity of the phosphatase enzyme. The significance of this phenomenon is discussed in greater detail elsewhere (p. 462).

Other Biliary Tract Conditions. Increased values for serum phosphatase activity have been reported in cases of biliary fistula in which all the bile was draining externally, a decrease occurring within ten days after correction of the fistula and the reappearance of bile in the intestine.²⁴ Many patients with portal cirrhosis present very high values for serum phosphatase activity with little or no increase in serum bilirubin concentration; others present normal phosphatase values with relatively high concentrations of serum bilirubin.^{4,5} Increased values for serum phosphatase activity may also be obtained in patients with metastatic carcinoma of the liver, the increase occurring in many instances before any demonstrable elevation of the serum bilirubin concentration. No increase in serum phosphatase activity has been found in cases of hemolytic jaundice.

MISCELLANEOUS CONDITIONS

Increased serum phosphatase activity has been reported in the following conditions: during periods of calcification of hemorrhages in scurvy, with subsequent return to normal as the calcified areas are absorbed; active tuberculosis; chronic myeloid leukemia; Hodgkin's disease involving the bones; Boeck's sarcoid. A decrease (below 45 units) has been reported in children with hypothyroidism.²³ Increased phosphatase activity has also been found in tumor cells.

ABNORMAL SERUM ACID PHOSPHATASE

Serum acid phosphatase determinations have been shown to be of clinical value only in carcinoma of the prostate with metastases.^{9,12,21} The source of this enzyme is the acinar epithelium of the prostate gland, which contains 500-2500 units per gram of fresh tissue (human and monkey) after puberty. A high concentration may be induced in the prostates of immature

rhesus monkeys by administration of androgens.⁶ Carcinomatous prostate tissue also contains large amounts of acid phosphatase, and when metastases occur, with invasion of lymph or blood channels, large amounts of the enzyme enter the circulation. Abnormally high values (above 4 units, and as high as 700 units per 100 cc.) have been obtained in about 85 per cent of cases of metastasizing carcinoma of the prostate and normal values in about 95 per cent of cases of nonprostatic disease, including skeletal and other disorders accompanied by high values for serum alkaline phosphatase. A slight increase (rarely over 6 and not over 10 units) may occur in advanced Paget's disease (osteitis deformans), osteopetrosis, hyperparathyroidism and carcinoma of the breast with extensive skeletal metastases.⁷ Care must be exercised to avoid hemolysis in the serum, which increases its acid phosphatase activity.

As stated by Gutman,⁷ normal values may be obtained in metastasizing prostatic carcinoma for the following reasons: (a) the prostatic cells may elaborate very little enzyme because of anaplasia or very low androgen levels; (b) there may not be sufficient invasion of the lymph or blood channels to permit entrance into the circulation of significant amounts of the enzyme; (c) depression of acid phosphatase activity may follow castration, administration of estrogens, intensive irradiation or radical prostatectomy.

For clinical purposes, serum acid phosphatase values below 3 units per 100 cc. may be regarded as negative and those over 10 units as positive in the diagnosis of metastasizing carcinoma of the prostate. A sharp drop follows successful application of the therapeutic procedures mentioned above, particularly castration and estrogen administration. Changes in this factor are of value in estimating the efficacy of treatment and in early detection of recurrence of metastases, and are therefore of prognostic value.

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Chapter VII

Magnesium Metabolism

ABSORPTION AND EXCRETION^{7,8,9}

THE absorption of magnesium from the bowel resembles that of calcium in many respects. An excessively high intake of fat, phosphate, calcium and alkalis appears to diminish the absorption of magnesium from the upper intestine. It is of interest also to note that a high magnesium intake appears to increase calcium elimination in the urine. The influence of the above-mentioned factors in this connection is probably dependent upon their influence on the solubility of magnesium salts. No extensive data are available regarding the effects of vitamin D upon magnesium absorption. The fact that the magnesium content of the bones is not decreased and is usually actually increased in rickets argues against the importance of vitamin D in this connection. However, the observations of Birk and Telfer suggest that vitamin D may be a factor in promoting absorption and retention of magnesium. Little or no such effects were noted more recently by Swanson.

Like calcium, magnesium is excreted in the feces and urine. Under normal conditions, about 50-80 per cent is excreted by the bowel (bile and intestinal secretion) and the remainder by the kidneys. After parenteral administration of magnesium salts, 70-90 per cent is excreted in the urine in the presence of normal renal function. The administration of acidifying substances, such as ammonium chloride and calcium chloride, is followed by an increased urinary elimination of magnesium, as of calcium.¹³ On the other hand, hyperthyroidism and thyroid administration, which cause an increase in urinary calcium, are practically without effect upon magnesium excretion.¹³ The urine magnesium increases slightly and temporarily after administration of parathyroid hormone, but then returns to normal.¹³ It is also within normal limits in clinical hyperparathyroidism. After correction of this condition by parathyroidectomy, there may be a temporary fall in magnesium excretion in the urine. There is evidence that the urinary excretion of magnesium is interfered with in the presence of renal functional impairment.

BLOOD MAGNESIUM

Magnesium is present in both red corpuscles and plasma. In contrast to calcium, the concentration in the red cells is apparently considerably higher than in the plasma. Perhaps because of variations in the methods employed and the limited number of cases studied, there is still disagreement regarding the normal range of variation in blood magnesium concentration. Recent studies indicate the normal limits of corpuscular magnesium to be 5.4 to 7.8 mg. per 100 cc., with an average of 6.6 mg.⁸ Previous studies yielded much lower values, the majority being about 1 mg. per 100 cc. The normal range of serum magnesium concentration is usually regarded as being from 2 to 3 mg. per 100 cc. Recent data indicate that values of 1.8 to 3.6 mg. per 100 cc. may be obtained in normal adults.⁸ Ultrafiltration and diffusion experiments indicate that 70 to 85 per cent (average 80 per cent) of the serum magnesium is in a diffusible state, the nondiffusible fraction being probably combined with the serum protein as in the case of the nondiffusible fraction of serum calcium. The magnesium content of cerebrospinal fluid is higher than that of blood serum, averaging about 3.3 mg. per 100 cc.

Little is known regarding the factors involved in the regulation of the magnesium content of the blood. It is relatively unaffected by phosphate, protein, vitamin D or parathyroid hormone, although the last apparently causes a slight and transient elevation of serum magnesium. There is in some respects a reciprocal relationship between magnesium and calcium in the serum; *e.g.*, in oxalate poisoning the decrease in serum calcium is accompanied by an increase in magnesium, while the hypermagnesemia induced by parenteral administration of magnesium salts is accompanied by a fall in serum calcium, at times to tetanic levels.⁴

Abnormal Serum Magnesium.⁵ Because of the occurrence of marked variation in red blood cells, studies of magnesium in the blood in pathologic states have been confined largely to the determination of its concentration in the serum.

The general opinion is that significant deviations from the normal do not occur consistently enough to be of distinct clinical importance. Slightly increased values have been reported in various chronic infections, atherosclerosis, hypertrophic arthritis, oxalic acid poisoning, essential hypertension and glomerulonephritis. Values as high as 13 mg. per 100 cc. have been reported following the ingestion of large quantities of magnesium sulfate by patients with chronic glomerulonephritis and renal functional impairment.⁶ It is believed by some that the degree

of magnesium retention under such circumstances may contribute to the development of coma which may be mistakenly regarded as uremic in nature. Many observers have failed to confirm these observations. However, it would appear that elevated values for serum magnesium may occur more consistently in chronic glomerulonephritis than in any other clinical disorder in which this factor has been studied. Values of 3 to 6 mg. per 100 cc. have been reported frequently. A slight and very transitory increase in serum magnesium concentration may occur following the injection of parathyroid hormone.^{3,13} This increase usually precedes demonstrable changes in calcium and phosphorus metabolism produced by this agent. Some observers have reported a decrease under the same conditions.

Low values for serum magnesium have been reported in uremia, normal and abnormal pregnancy, rickets, hypervitaminosis B, marked experimental magnesium deprivation, a form of tetany unassociated with disturbances in calcium and phosphorus metabolism, and in epilepsy. However, many of the figures regarded by individual authors as subnormal fall within the range regarded as normal by others. In fact, with few exceptions, the same statement may be made regarding the great majority of all reported abnormal values for serum magnesium concentration.

An increase in the nondiffusible fraction of serum magnesium (25-60 per cent of the total) has been reported in hyperthyroidism, normal values (15-30 per cent of the total) being restored by adequate treatment with iodine or subtotal thyroidectomy. Very low values for nondiffusible magnesium, below 5 per cent of the total, and at times 0 per cent, have been obtained in patients with myxedema, an increase occurring after administration of thyroid extract.^{2,10} The significance of these changes is not known.

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Chapter VIII

Metabolism of Iron¹⁻⁸

ABSORPTION AND EXCRETION

ALTHOUGH the total amount of iron in the body is extremely small, about 45 mg. per kilogram of body weight, the fact that it is an essential constituent of hemoglobin and of chromatin makes it an element of great importance in fundamental processes of nutrition. Its chief functions lie in the transport of oxygen to the tissues (hemoglobin) and in cellular respiration or oxidative mechanisms (cytochrome). The formerly prevalent opinion that inorganic iron is absorbed but slightly or not at all and that the iron in foodstuffs is present only in complicated organic form and is alone assimilated for hemoglobin production is no longer tenable. The degree of absorption of inorganic iron salts compares favorably with that of iron in foods, and it would appear that the factors involved in limiting the absorption and utilization of iron are concerned not so much with the form in which iron is ingested as with some unknown intra-intestinal factors (Hahn). Hematin iron is not available for utilization or 'hemoglobin production' by the animal organism.

According to Hahn, a number of factors may make absorption of iron difficult regardless of the form in which it is administered: (a) The relatively high pH in the jejunum facilitates the formation of insoluble basic iron compounds; (b) The alkalinity of pancreatic juice and the relative insolubility of the iron salts of bile acids probably interfere with the absorption of iron; (c) Absorption of iron appears to be interfered with in the absence of free HCl and of bile, and is also influenced unfavorably by the administration of alkalis; (d) The presence of relatively large quantities of phosphate facilitates the formation of insoluble iron phosphate compounds, which are poorly absorbed. It is believed that absorption of this element occurs chiefly in the stomach and upper duodenum. Theoretically, ferrous salts may be absorbed more readily than ferric salts, since there are fewer possibilities of the formation of unabsorbable compounds. Absorption may be decreased by increased intestinal motility (diarrhea).

There is evidence that, in experimental animals at least, the normal organism absorbs iron only in proportion to its needs, the quantity absorbed being determined by the magnitude of the body reserves of this element.⁴ It would appear that the intestinal mucosa is the tissue responsible for its acceptance or rejection, being perhaps conditioned by the iron content of the blood, anemic animals absorbing and utilizing iron very efficiently.⁶

Comparatively little iron is excreted from the body, the bulk of the fecal iron comprising that which has escaped absorption from the intestine. The small amount actually excreted is eliminated chiefly in the bile, to a lesser extent by the intestinal mucosa and to a slight extent by the kidneys.

BLOOD IRON

Iron is present in the blood in organic and inorganic forms. The iron content of whole blood ranges normally from 40 to 60 mg. per 100 cc., averaging 45 in females and 52 in males. Practically all of this is in organic form as hemoglobin, which contains about 0.335 per cent iron. The inorganic iron content of whole blood averages about 1-1.7 mg. per 100 cc. Blood plasma contains a small amount of inorganic iron, 0.084-0.17 mg. per 100 cc., which appears to be in ferric form. This value is quite constant in the fasting state, representing iron in the process of transportation and is probably an index of the activity of the intermediary metabolism of iron, particularly hemoglobin destruction and formation. About 90 mg. of iron are liberated daily by normal disintegration of erythrocytes.

After ingestion of inorganic iron, the plasma iron concentration increases three- to fourfold in about two to four hours, falling subsequently over a period of six to twelve hours, depending upon the amount ingested. The plasma appears to be the medium of transportation of iron from the intestine to the tissues in which it is utilized and stored. Absorbed iron is transferred to erythrocytes with remarkable rapidity, radioactive iron having been demonstrated in red blood cells in anemic dogs within a few hours after its ingestion.⁶ The plasma iron is influenced by and may be regarded as an index of (a) the quantity of iron absorbed from the intestine, (b) the adequacy of the tissue iron reserves, (c) the capacity of the bone marrow to utilize iron for hemoglobin synthesis and (d) the activity of hemolytic processes.⁵

INTERMEDIARY METABOLISM

Iron is probably absorbed in the form of ferrous salts and is transported in the plasma as ferric iron. The endogenous supply

of iron, about 90 mg. daily, representing the breakdown of about 29 Gm. of hemoglobin, far exceeds the exogenous supply in the food, and practically all of it is retained in the body, even under conditions of excessive hemolysis. During the course of destruction of erythrocytes, hemoglobin is decomposed into globin (protein) and hematin, and the latter into iron-containing hemosiderin and a series of iron-free pigments terminating in bilirubin (p. 430), which is excreted in the bile. The iron thus liberated is partly stored or reconverted to hemoglobin in situ,

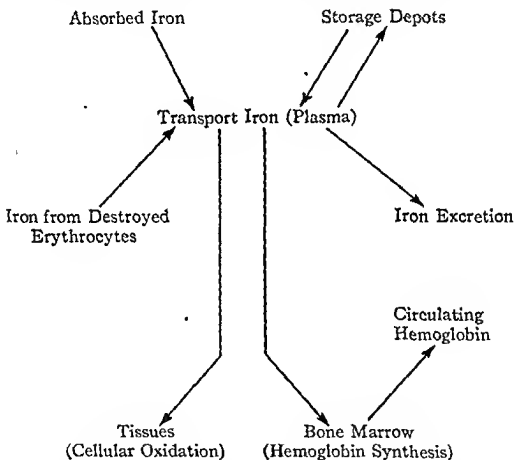


Fig. 4.—Intermediary metabolism and storage of iron (After Moore, Doan and Arrowsmith)

or is transported in the plasma (a) to storage depots, (b) to the bone marrow for hemoglobin synthesis or (c) to the tissues for participation in processes of cell respiration.

The exogenous iron requirement of normal adults is believed to be 5–15 mg. daily. This amount is apparently adequate to meet the demands of normal menstruation, pregnancy and lactation, although 15–20 mg. is probably more satisfactory in the latter states. Young children (four to eight years) require about 0.6 mg. and infants (to one year) 1–2 mg. per kilogram of body weight.

Following its absorption, iron is carried to the liver, where most of it is removed from the blood, and then to the other body tissues, where it apparently exists in two functionally different forms, designated (a) parenchyma iron and (b) available storage iron. The distribution of the 3 Gm. or less of iron in the body has been estimated as follows: (1) blood hemoglobin iron, 57 per cent of total; (2) muscle hemoglobin iron, 7 per cent; (3) parenchyma iron (muscle and other tissues), 16 per cent; (4) available tissue storage iron (liver, spleen, marrow), 15 per cent; (5) available iron of other tissues, 5 per cent. Thus, about 65 per cent of the body iron is combined as hemoglobin, 15 per cent as functional iron of the tissues and 20 per cent stored in available forms. Muscle hemoglobin and parenchyma iron are unavailable for blood hemoglobin production and are not drawn upon, no matter how great the emergency due to anemia. There is evidence that blood hemoglobin may constitute an important source of iron necessary for growth. In the presence of increased demand (anemia, inadequate intake), iron is mobilized readily from tissue depots.

ABNORMAL IRON METABOLISM

Disturbances in iron metabolism are evidenced by (a) decreased formation of hemoglobin, (b) decrease in circulating hemoglobin, (c) abnormalities in the serum iron concentration or (d) abnormal deposition of iron-containing pigment (hemosiderin) in the tissues. A consideration of the pathogenesis of various types of anemia is beyond the scope of the present discussion, but it may be stated that certain forms of hypochromic and microcytic anemia are dependent primarily upon inadequate supply or absorption of iron, the latter occurring particularly in the presence of gastric anacidity. Iron deficiency may also result from hemorrhage, with exhaustion of the available tissue reserves of this element. Low values for plasma or serum iron have been reported in hemorrhagic and hypochromic types of anemia. High values occur in forms of anemia characterized by diminished hemoglobin formation not due to iron deficiency, as in pernicious anemia.

Deposition of excessive amounts of hemosiderin in the tissues occurs (a) as a result of excessive breakdown of erythrocytes in hemolytic types of anemia, (b) in conditions in which there is inadequate synthesis of hemoglobin due to factors other than iron deficiency (pernicious anemia) and (c) in hemochromatosis, the cause of which is unknown. In the last condition, enormous amounts of iron may be deposited in the tissues, especially the liver, pancreas and retroperitoneal lymph nodes,

over 50 Gm. having been found in the body (exclusive of the blood) in some cases.^{7,8} Studies of iron balance have revealed no significant abnormality in this condition, but it is possible that a slight degree of abnormal retention may exist over a period of many years.

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Chapter IX

Sulfur Metabolism^{1,2,6-9}

SULFUR is a nutritionally essential element, occurring in substances of great physiologic importance, including thiamine, insulin, anterior pituitary hormones, glutathione, cysteine, methionine, etc. However, indications for and methods of investigation of disturbances of sulfur metabolism are so limited that this subject requires only brief discussion here.

ABSORPTION

Sulfur is ingested in (1) inorganic (NaCl and Mg sulfates) and (2) organic forms. The latter may be subdivided into (a) nonprotein sulfur (sulfolipids and sulfatides) and (b) protein sulfur (the sulfur-containing amino acids, cystine and methionine; glycoproteins, such as mucoitin-sulfuric acid in mucin and ovomucoid and chondroitin-sulfuric acid in cartilage and tendons). The most important source of sulfur is the cystine and methionine of ingested proteins. Inorganic sulfate is absorbed as such from the intestine into the portal circulation, as are the amino acids, cystine and methionine, liberated by digestion of protein. A small amount of sulfide may be formed in the bowel by the action of putrefying bacteria but, if absorbed into the blood stream, this is rapidly oxidized to sulfate.

INTERMEDIARY METABOLISM

Sulfur reaching the liver in the portal blood undergoes the following changes:

(1) A portion of the organic S escapes oxidation. Some of this fraction is utilized for the formation of S-containing substances, such as insulin, anterior pituitary hormones, taurocholic acid, melanin, glutathione. The remainder is excreted in the urine as neutral S.

(2) The bulk of the organic S is oxidized in the liver to inorganic sulfate. A portion of the latter, together with a portion of the inorganic sulfate absorbed from the intestine, enters the systemic circulation and is excreted in the urine.

(3) A portion of the inorganic sulfate is combined in the liver with various phenol derivatives, such as indol, formed in

the bowel largely by bacterial decomposition of protein, to form ethereal sulfates, which are excreted in the urine. In addition to the participation of inorganic sulfate in this detoxication process, the amino acid cysteine (formed from cystine) may form conjugation products (mercapturic acids) with certain toxic compounds, such as brombenzene.

SULFUR IN BLOOD

The normal concentration of sulfur in the blood serum is as follows:⁴ inorganic S, 0.5–1.1 mg. per 100 cc.; ethereal sulfate S, 0.1–1.0 mg. per 100 cc., neutral S, 1.7–3.5 mg. per 100 cc. Whole blood contains 2.2–4.5 mg. of neutral S per 100 cc., the higher value being due in large measure apparently to the presence of thioneine and glutathione in the blood cells. The serum sulfate concentration may be increased in the presence of renal functional impairment, pyloric and intestinal obstruction and leukemia. Marked sulfate retention in advanced glomerulonephritis may contribute to the development of acidosis. An increase in the blood indican concentration (indoxyl potassium sulfate) may occur in uremia (normal, 0.026–0.085 mg. per 100 cc.).

EXCRETION

Sulfur is excreted in the urine in the three forms in which it exists in the blood. The total S output, since it is derived chiefly from protein under ordinary circumstances, varies with the protein intake and the extent of tissue protein catabolism, averaging about 2.5 Gm. (as SO_3) daily under normal conditions. The urinary N:S ratio normally ranges from 13 to 16 in the fasting state or when meat constitutes the bulk of the protein intake.

The normal total sulfate content (inorganic plus ethereal sulfates) of the urine on an average mixed diet ranges from 1.5 to 3 Gm. daily (as SO_3), comprising 85–95 per cent of the total S output. The proportion as well as the absolute amount excreted varies directly with the protein intake. The total sulfate excretion may be diminished in the presence of renal functional impairment and is increased in conditions accompanied by excessive tissue protein breakdown, such as high fever and increased metabolism.

Inorganic sulfate (Na, K, Ca, Mg and NH_4 sulfates) normally comprises 85–95 per cent of the total sulfate of the urine, the remainder (5–15 per cent) being ethereal sulfate, which normally ranges from 0.1 to 0.25 Gm. daily (as SO_3). This fraction consists of sodium and potassium salts of phenolic

sulfuric acid compounds (e.g., indoxyl potassium sulfate or indican) and therefore varies with the quantity of phenolic substances produced in the intestine or otherwise entering the body. Increase in urine ethereal sulfate (indican) (absolute and relative) may occur in intestinal obstruction, (intestinal stasis and increased putrefaction), paralytic ileus, generalized peritonitis, typhoid fever, and in association with bacterial decomposition of tissue protein and purulent exudates, as in gangrene, empyema, pulmonary suppuration, tuberculosis with cavitation and secondary infection. The greatest clinical importance of urine sulfate partition studies lies in the demonstration by this means of abnormal absorption of benzene and the control of industrial exposure to this agent.^{2,5,10} Benzene is excreted in the urine in the form of an ethereal sulfate (conjugated in the liver), the absorption of abnormally large amounts being reflected in a decrease in the proportion of total sulfate excreted as inorganic sulfate (below 70-80 per cent of the total urine sulfate). In acute cases these values may fluctuate rather rapidly, e.g., from 8-10 per cent immediately after exposure to 75-80 per cent two days later.

The neutral S of the urine, normally comprising about 5 per cent of the total sulfur, consists of such substances as cystine, urochrome, taurine and its derivatives, oxypurine acids, thiocyanate and thiosulfate. This fraction is increased in cystinuria (p. 125), melanuria (p. 126) and some cases of obstructive and hepatocellular jaundice (excretion of taurocholic acid in the urine) (p. 453).

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Chapter X

Iodine Metabolism

THE metabolism of iodine is of particular significance in connection with the functional activity of the thyroid gland. This subject has acquired clinical importance in recent years because of the development of chemical methods for the detection and quantitative estimation of minute quantities of iodine in small amounts of biologic material, including blood. It has been found that the iodine requirement of the normal human adult does not exceed 25 micrograms daily (1 microgram or gamma = 0.001 mg.).³ It seems advisable, however, to place the optimal daily intake at 100-200 micrograms for adults, 50 for children and 20-40 for infants. The requirement is probably increased during pregnancy. There are apparently distinct differences in the availability of iodine supplied in different forms. However, that of most common foods appears to be readily available to the organism. There is no evidence that food iodine is superior in this respect to inorganic iodine.

ABSORPTION AND EXCRETION^{4a, 12a, 17, 17a}

Normal. Iodine and iodides can be absorbed from any portion of the alimentary tract but most readily perhaps from the small intestine, free iodine and iodates probably being first converted to iodides. Organic iodine compounds, as diiodotyrosine and thyroxine, are in part absorbed as such and in part broken down in the stomach and intestine with the formation of iodides. Absorption can occur from other mucous membranes, the lungs and the skin.

Iodine is excreted by the kidneys, liver, intestine, skin and lungs, and also in milk and saliva. Under ordinary circumstances it is almost entirely inorganic, and may be either endogenous (mobilized from stores in the body or from destruction of thyroxine or diiodotyrosine) or exogenous (food, water, air). No thyroxine is demonstrable in excreta (except after administration) and the question of the presence of diiodotyrosine is unsettled.

The quantity excreted under normal conditions naturally varies with the intake. About 40-80 per cent of the total is

ordinarily eliminated by the kidneys, the usual daily range being about 20-70 micrograms (gamma) in adults and 20-35 in children. It is almost entirely in the form of inorganic iodide and, perhaps, at times a small amount of diiodotyrosine, being chiefly endogenous. The proportion excreted in the urine is greatest when the intake is lowest, diminishing as the amount ingested increases. Urine iodine is increased by exercise and other factors that increase metabolism, except in the event of profuse sweating, when the amount in the sweat is greatly increased. Urine iodine also increases during pregnancy. After administration of iodides, the excess is eliminated chiefly in the urine, the most marked increase occurring during the first six hours and the remainder being lost in diminishing amounts during the next four days.

Iodine excreted in the feces is almost entirely exogenous, consisting of that ingested which has passed through the intestine unabsorbed and that eliminated in the bile and intestinal secretions. It varies normally from 2 to 11 gamma daily, constituting 3-27 per cent of the intake. It falls to 0-0.4 gamma after a forty-eight hour fast and increases with a high intake or during periods of diarrhea. The bile contains about 4-14 gamma per 100 cc. in the resting state and an average of 50 gamma per 100 cc. after eating. A portion of this is reabsorbed in the intestine, constituting an enterohepatic circulation of iodine. Iodine in the bile is chiefly of alimentary origin but is also in part endogenous, since it does not disappear during fasting periods. After intravenous injection of thyroxin in large doses, some may be excreted unchanged by the intestine. The concentration of iodine in the saliva may vary from 0 to 350 gamma per 100 cc.

At high altitudes, low temperatures and low humidity, the amount of iodine eliminated by the skin is negligible. With profuse perspiration (heat, humidity, exercise), as much as 30-60 per cent of the total may be excreted by this route (0-68 gamma daily). The quantity in the expired air varies enormously, but may be as much as 10-30 gamma daily. The amount present in human milk is important because of its obvious relation to the requirement of the newborn infant. Colostrum, on the first day, contains 8-40 gamma per 100 cc., and on the fifth day 2-3 gamma. As is true of cow's milk, this depends upon the iodine intake and may be increased by administration of iodine. It is practically entirely in inorganic form.

Abnormal. The increased level of blood iodine in hyperthyroidism (p. 218) is accompanied by a corresponding increase in its excretion by the kidneys, liver, intestine and skin.^{17, 17a, 19}

The daily urine iodine may reach 40-950 gamma, depending upon the intake and the concentration in the blood. In some cases a negative balance of as much as 200 gamma daily has been observed. Under such circumstances, the body reserve of 20-50 mg. would be exhausted in a short time, but it is probable that the blood iodine concentration and its excretion decrease before this occurs.

Increased excretion of iodine in the urine has been observed during menstruation and pregnancy, in certain acute infections (also in perspiration), during periods of excitement, immediately following partial thyroidectomy in some cases of hyperthyroidism⁴ and after a variety of surgical procedures. Whether or not this phenomenon is dependent upon increased thyroid activity is debatable.

In myxedema, the urine iodine usually decreases more markedly in the fasting state than in normal individuals. Under ordinary conditions of normal iodine intake, little if any deviation from the normal excretion can be observed, since this depends largely upon the supply. However, it has been stated that after ingestion of iodine, the increase in urinary excretion in myxedema is greater and more prolonged than normal. This is perhaps related to the inability of the atrophic thyroid to take up iodine as completely as the normal gland.

BLOOD IODINE

Normal values for blood iodine (fasting) have been reported ranging from 2 to 20 gamma per 100 cc. Recent refinements in chemical technics have resulted in lower values within a much narrower range. The normal blood iodine concentration probably does not vary beyond 2.4-5.5 gamma per 100 cc.^{2,11,13,15} In the absence of previous iodine administration, practically all of the blood iodine is in the plasma or serum, which contains 4-10 gamma per 100 cc.^{11,13,16} and which seems to be the best medium for clinical determinations of blood iodine.

Under normal resting conditions, if no iodine has been administered during the preceding ten days, less than 30 per cent of the plasma iodine is in inorganic form, the remainder being bound to protein, chiefly albumin, constituting the so-called "organic" iodine fraction,^{13,17} which ranges from 4 to 8 gamma per 100 cc. In normal subjects, about 60-75 per cent of the "organic" iodine fraction represents a substance or substances behaving like thyroxin, the remainder resembling diiodotyrosine. It appears that the circulating form of the thyroid hormone differs from the storage form (iodothyro-

globulin), which has not been demonstrated in the blood, even in thyrotoxicosis.

Fluctuations in protein-bound or "organic" iodine are due almost entirely to variations in the thyroxin-like fraction, being very low in myxedema (0-3 gamma) and high in thyrotoxicosis (8-30 gamma), and may be assumed to represent variations in the quantity of circulating thyroid hormone. This factor is therefore of value in measuring the degree of thyroid activity in cases in which the diagnosis cannot be made on the basis of other findings.¹⁸

The blood iodine increases after ingestion of iodine, is somewhat higher in summer than in winter (denied by some), varies with geographical situation (intake) and apparently increases slightly after fifty years of age (climacteric?). A sharp rise occurs within a few minutes after vigorous exercise, with a return to normal after about two hours of rest. An increase may occur at times during the latter half of pregnancy, in labor, and on the first day of menstruation.

In the fasting state, the plasma inorganic iodine is quite constant despite wide variations of thyroid activity, but increases strikingly after ingestion of iodine, inhalation of iodine vapors, application of iodine to the skin, cholecystography or intravenous urography (employing iodine compounds). Values as high as 2000 gamma per 100 cc. have been obtained after applying tincture of iodine to the skin preparatory to lumbar puncture.¹ The normal level may be restored within two but occasionally not until ten days after discontinuing administration of iodine in nonhyperthyroid subjects.

Ingestion of thyroglobulin, thyroid substance or thyroxin is followed in about two hours by a simultaneous and practically equal increase in both "organic" and inorganic fractions in the blood. This is believed to be due to disintegration of a portion of these substances in the intestine or liver, with consequent entrance of both forms of iodine into the circulation. When thyroxin is given intravenously, the increase in blood iodine occurs only in the "organic" fraction, which returns to the pre-injection level in about two hours.

An increase in blood iodine has been reported in about 40 per cent of cases of lymphatic leukemia and occasionally, according to some observers, also in myeloid leukemia. This increase is not related to the basal metabolic rate. Conflicting findings have been reported in other conditions accompanied by elevated basal metabolic rates, *viz.*, essential hypertension and congestive heart failure, but the reported increased values refer to total iodine and it seems probable that "organic" iodine deter-

minations will yield more consistently normal findings in these conditions. Elevated total iodine values have been observed in obstructive and hepatocellular jaundice, in occasional cases of lymphosarcoma, carcinoma of the breast, stomach and kidney, severe infections, during periods of remission in pernicious anemia and after a variety of surgical procedures.

INTERMEDIARY METABOLISM

The body normally contains about 20-50 mg. of iodine, the bulk of which is distributed approximately as follows: muscles, 50 per cent; thyroid, 20 per cent; skin, 10 per cent; skeleton, 6 per cent. It is present in much higher concentration in the thyroid (10-40 mg. per 100 Gm.) than in other tissues (0.03 mg. per 100 Gm. in muscle). The quantity in all tissues diminishes when the intake is lowered, but the normal thyroid retains its capacity for trapping and storing iodine even under such circumstances. The normal adult body contains about 12-14 mg. of thyroxin.

The liver usually contains 500-2300 gamma of iodine, with a marked variation during periods of absorption and excretion. Iodine entering the portal vein from the intestine in part passes through the liver into the systemic circulation and in part is excreted in the bile. Organic iodine, exogenous or endogenous, is partially or completely broken down in this organ. After administration of inorganic iodine, apart from the enormous increase in the thyroid, temporary storage (a few days) occurs in the liver, kidneys, skin, heart and lungs.

The normal, adult thyroid gland contains 2-28 mg. of iodine, the concentration ranging from 0.1 to 0.55 per cent of dry weight. It is present practically entirely in the colloid and varies with the iodine intake, geographical location (intake), season and state of endocrine function. The quantity increases from birth to puberty, reaching a maximum at about twenty years and usually decreasing after fifty years of age. The higher content in maritime regions is attributed to the larger amount of iodine in the water, soil and air.

By far the largest part of the thyroid iodine is in organic form. It seems probable that the thyroid "hormone," iodothyroglobulin, is a composite molecule, containing thyroxin and diiodotyrosine in peptide combination. About 25-35 per cent is in the form of thyroxin or thyroxin-like substances, and 60-65 per cent is in a form resembling diiodotyrosine. There is evidence that thyroxin may be formed from tyrosine through the intermediate stage of diiodotyrosine, synthesis of these substances occurring in the thyroid gland. It has been estimated that about

0.33 mg. of thyroxin is liberated into the blood every twenty-four hours. The remarkable affinity of the thyroid colloid for iodine is evidenced by the fact that the normal gland is surfeited with new iodine within fifteen minutes after administration of radioactive iodine.⁹ The added iodine is quickly incorporated in the thyroid protein molecule, being perhaps transformed progressively into diiodotyrosine and thyroxin. A portion, depending upon the storage capacity of the gland at the moment, diffuses back into the blood, so that little or no inorganic iodine remains in the gland after twenty-four to forty-eight hours. However, an "iodine-starved" gland may retain 10-20 per cent of a single therapeutic dose and after repeated doses, the iodine content may increase to over 1 per cent of the dry weight. Secretion of thyroid hormone varies in response to nervous (sympathetic) and hormonal (thyrotrophic) stimulation. Thus the thyroid gland regulates iodine metabolism in two ways: (1) it fixes iodine by incorporating it within the organic molecule of iodothyroglobulin and (2) it releases portions of this molecule into the circulation in accordance with the physiologic needs of the organism. By virtue of these properties it maintains the blood iodine within normal limits.

The relatively high concentration of iodine in the anterior hypophysis is of interest because of the relation of the thyrotrophic principle elaborated by this gland to thyroid function. Administration of this substance causes hyperplasia of the thyroid gland, with increased mitosis and decreased colloid. These changes are accompanied by manifestations of thyrotoxicosis, increase in iodine in the blood and urine, and decrease in the iodine content of the thyroid. After hypophysectomy, the blood iodine increases for about two weeks and then falls gradually, with similar changes in urine iodine. The iodine content of the ovary, 30-250 gamma per 100 Gm. in adults, is lower before puberty and after the menopause. The changes in blood iodine that occur during menstruation and pregnancy may be dependent upon primary ovarian or pituitary influences.

In myxedema, the thyroid gland is atrophic and its iodine content is decreased, as is the capacity of the gland for taking up exogenous iodine. In hyperthyroidism, the iodine content of the thyroid is decreased, approximately in proportion to the decrease in colloid and the degree of hyperplasia. The iodine concentration falls below 0.1 per cent of the dry weight and may be as low as 0.02 per cent. This is due to inability of the gland to store iodine (thyroid hormone) under existing conditions and not to decreased formation of thyroid hormone. The increase in circulating organic iodine is the result of this impaired storage

capacity. That the gland has not lost its ability to take up iodine in hyperthyroidism is indicated by the phenomenon that follows administration of a comparatively large amount of iodine, as in the iodine tolerance test.

Iodine Tolerance Test. After withdrawing a control blood sample, 6 minims of Lugol's solution (37 mg. I) are taken in a little milk and blood samples are obtained for iodine determination at half-hour intervals for two and one-half hours subsequently. In nonthyrotoxic subjects, the blood iodine concentration rises to over 80 gamma per 100 cc. (usually over 100 gamma) at one-half-hour and remains at this level, or at least considerably above the resting level, during the test period. In hyperthyroidism, the rise in blood iodine is relatively slight, the maximum being less than 80 gamma per 100 cc. It has also been found that intravenously injected iodine (250 gamma per kilogram) disappears from the blood more rapidly than in normal subjects. It has been shown that this is not due to increased excretion but rather to increased affinity of the thyroid for added iodine. This has been demonstrated by using radioactive iodine as a tracer.^{7,8,10}

These findings throw some light upon the disturbance of iodine metabolism in hyperthyroidism. They indicate the presence of an actual iodine deficiency in this condition, the gland being capable of storing iodine if an excess is given. The underlying cause of the impaired storage of iodine in the hyperplastic thyroid gland is not known, but it results in an increase in blood iodine with a consequent increase in iodine excretion. Normal blood iodine values in hyperthyroidism have been attributed to prolonged excretion of increased amounts with depletion of the body iodine stores.

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Chapter XI

Sodium, Potassium and Chloride Metabolism

THE metabolism of these substances is intimately related to the water balance and acid-base equilibrium of the body, which are considered in detail elsewhere (pp. 248, 265) and which will therefore be mentioned here only briefly. Sodium, potassium and chloride are concerned in at least four fundamental physiologic processes: (1) the maintenance of normal water balance and distribution; (2) the maintenance of normal osmotic equilibrium; (3) the maintenance of normal acid-base equilibrium (physiologic neutrality); (4) the maintenance of normal muscle irritability. The maintenance of normal hydration and osmotic pressure depends primarily upon the total base content of the body fluids (p. 253). Since sodium constitutes the largest fraction of the total base of the extracellular fluids ($\frac{142}{155}$), it plays a dominant role in this connection (p. 254). At any given H_2CO_3 concentration, the hydrogen ion concentration of the plasma and other extracellular fluids depends upon the bicarbonate concentration $\left(H^+ = K \frac{H_2CO_3}{BHCO_3} \right)$. Since the bicarbonate

content depends upon the amount of total base present in excess of anions other than H_2CO_3 , and since Cl constitutes by far the largest fraction of the total anions of the plasma ($\frac{103}{155}$), it naturally follows that the maintenance of the normal pH depends largely upon the presence of normal concentrations of sodium and chloride (p. 253). The importance of a proper balance between Na, K, Mg and Ca in the maintenance of normal neuromuscular irritability and excitability is well known. It may be expressed as follows:

$$\text{Irritability} \propto \frac{[Na^+] + [K^+]}{[Ca^{++}] + [Mg^{++}] + [K^+]}$$

Potassium is the chief cation of the muscles and of most other cells (intracellular fluid), whereas sodium is the chief cation of extracellular fluids of the body. Although some movement of potassium and water occurs from cells to plasma, particularly when excessive amounts of sodium chloride and

water are lost from the body, and in disturbances of acid-base balance, the potassium is usually excreted promptly in the urine. Any considerable replacement of sodium by potassium in the extracellular fluids is accompanied by serious disturbances and is eventually fatal. Moreover, no other cation can entirely replace potassium in the intracellular fluid without interfering to a certain extent with the functional activity of the cell. In low concentrations potassium is excitatory and in higher concentrations it is inhibitory,* these effects being particularly important in relation to nerve synapses or myoneural junctions. Under normal conditions, its effects resemble those of parasympathetic stimulation, and are usually inhibited by calcium (e.g., neuromuscular excitability). The K/Na ratio is also important in this connection. Potassium and calcium modify the most fundamental properties of protoplasm and cells, including the permeability of cell membranes, and thus they play a role in almost all "vital" processes.

NORMAL SODIUM, POTASSIUM AND CHLORIDE IN BLOOD

The concentration of electrolytes in the blood serum may be expressed either in terms of milligrams per 100 cc. or milliequivalents per liter; the latter may be calculated from the former according to the formula:

$$\text{Milliequivalents per liter} = \frac{\text{milligrams per liter} \times \text{valence.}}{\text{atomic weight}}$$

Calculation of electrolyte concentration in terms of milliequivalents is commonly employed because it facilitates the expression of normal and abnormal relationships between the acid and base constituents of the body fluids. In fluids as neutral in reaction as the body fluids, the basic elements must be almost completely combined with acid radicals as neutral salts. The sum of the acid equivalents must, therefore, be equal to that of the base equivalents, and the total electrolyte content of body fluids is determined by their total base content. In terms of milliequivalent concentration, normal blood serum presents approximately the following distribution of acid and base elements:

Total Base = Na + K + Ca + Mg equals
 155 142 5 5 3

Total Acid = Cl + HCO₃ + PO₄ + Protein + SO₄ and Organic Anions
 155 103 28 2 16 6
 (the numbers represent milliequivalents per liter of serum)

The internal structure of the anion pattern may vary considerably in pathologic states with no significant change in the

total base concentration. For example, as stated by Peters, loss of HCl by prolonged vomiting may result in the disappearance of half of the chloride and its replacement by HCO_3 and other anions; short vigorous exercise may result in the replacement of much of the bicarbonate by lactate; hyperventilation may result in diminution in bicarbonate and a corresponding increase in chloride or organic anions; the accumulation of organic acids during periods of starvation or in diabetic acidosis results in a decrease in bicarbonate and chloride. However, in the majority of instances, the total milliequivalent concentration of the blood plasma remains within normal limits. This is generally regarded as an indication of the fundamental importance of the maintenance of the normal osmotic pressure in the body fluids, even at the expense of marked changes in the concentration of individual electrolytes. A decrease in total base does, however, occur under certain circumstances, notably in terminal stages of renal disease, diabetic acidosis and prolonged diarrhea or vomiting and in severe adrenal cortical insufficiency (Addison's disease).

Normal Blood Sodium. The concentration of sodium in normal blood serum ranges from 315 to 340 mg. per 100 cc., averaging about 330 mg.; these values correspond to a range of approximately 137 to 147 milliequivalents per liter, averaging 143. There is little or no sodium in the red corpuscles.

Normal Blood Potassium. The potassium concentration of normal blood serum is 16-22 mg. per 100 cc., averaging 19 mg.; these values correspond to a concentration of 4.1-5.6 milliequivalents per liter. While there is little or no sodium in the erythrocytes, the average concentration of potassium in these cells is about 420 mg. per 100 cc.

Normal Blood Chloride. The chloride content of whole blood normally ranges from 450 to 500 mg. per 100 cc. (as sodium chloride), the normal values for plasma being 570 to 620 mg. per 100 cc. The actual chloride content of whole blood is 270-300 mg. per 100 cc., that of plasma or serum being 340-370 mg. per 100 cc. or 96-105 milliequivalents per liter. The distribution of chloride in the plasma and red cells is similar and intimately related to that of HCO_3 , in accordance with Donnan's law governing the distribution of diffusible monovalent ions (p. 230). The fact that the red cells contain only about one-half as much chloride as the plasma is due to their relatively high protein (hemoglobin) content and their relatively low water content, the former factor limiting the quantity of base available for combination with electrolytes and ions while the latter factor limits the concentration of contained electrolytes compatible

with the maintenance of isotonicity. The factors which alter the distribution of chloride between the plasma and red cells are considered in detail in the section dealing with the acid-base equilibrium. Suffice it to state here that an alteration in the concentration of either Cl or HCO_3 in the plasma is associated with a reciprocal change in the other factor; *i.e.*, an increase or decrease in the concentration of chloride (plasma) is accompanied by a corresponding decrease or increase in the plasma bicarbonate concentration, and vice versa. This physicochemical equilibrium plays an important part in the maintenance of the normal acid-base balance of the blood (see p. 253).

Chloride is present in all cells and body fluids, and it is generally assumed that it is readily diffusible through cell membranes. However, there is some evidence that this is not true of muscle cells. Its concentration is slightly higher in the lymph and serous fluids than in the plasma, its concentration in these fluids varying roughly inversely as their protein content.

It is obvious that because of this unequal distribution of chloride in the blood the chloride content of whole blood varies considerably with variations in the number of red cells, being relatively high in anemia and relatively low in polycythemia. For this reason blood chloride determinations in clinical conditions should be made upon plasma rather than whole blood. Because of the shift of chloride from plasma to red cells, and in the opposite direction, with changes in the HCO_3 concentration of the plasma (see p. 271), blood drawn for plasma chloride estimations should be collected under oil, for, if exposed to air, CO_2 escapes from the blood, the chloride content of the plasma being thereby increased. Furthermore, the blood should be centrifuged and the plasma separated as soon as possible to avoid the shift of chloride from plasma to red cells incident to the formation of acid which occurs in the blood upon standing.

In the normal individual the plasma chloride undergoes but slight physiologic variation. It has been observed to fall slightly during periods of active gastric secretion and to rise in the post-digestive period owing probably to the reabsorption of chloride from the intestine. The administration of excessively large quantities of salt to normal individuals causes only a slight increase in the concentration of plasma chloride, and the complete withdrawal of salt from an otherwise normal diet rarely causes a diminution in plasma chloride below the low limit of normal (570 mg. per 100 cc., as sodium chloride). In the fasting individual, however, the plasma chloride concentration falls below normal, due in part to sodium chloride deprivation but chiefly perhaps to the fact that the acidosis resulting from

starvation causes the passage of chloride from the plasma to the red cells (see acid-base balance, p. 280).

ABSORPTION AND EXCRETION^{11,20,25}

Under conditions of normal gastro-intestinal function, sodium and potassium are practically completely absorbed from the gastro-intestinal tract. Normally, less than 2 per cent of the sodium ingested and less than 10 per cent of the potassium are eliminated in the feces; large quantities are eliminated in the feces in patients with diarrhea, but this excess may be

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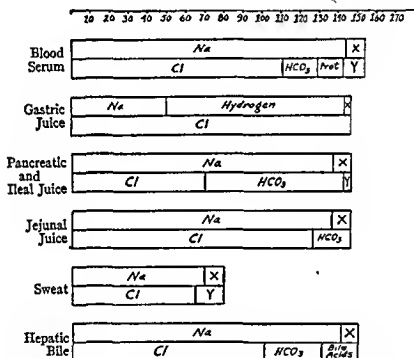


Fig. 5.—Electrolyte composition of body fluids X = unnamed basic radicles. Y = unnamed acid radicles. (After McCance.)

due in large part to failure of reabsorption of digestive fluids rather than entirely to failure of absorption of the ingested salts.

Under normal conditions, more than 95 per cent of the excreted sodium and about 90 per cent of the excreted potassium are eliminated in the urine. Sodium is also the most important base of all normal extracellular fluids of the body, with the exception of gastric juice (hydrogen). Consequently, excessive loss of such fluids, particularly gastro-intestinal secretions, urine and sweat, may result in profound disturbances in sodium metabolism and, because of their intimate relationship with the latter, chloride metabolism and water balance (p. 255). The important electrolyte relationships in the body fluids are presented in Fig. 5.

Because of the fact that by far the greatest portion of the body sodium exists in the form of sodium chloride, abnormalities of sodium balance and concentration in the serum are usually accompanied by simultaneous and similar alterations in chloride balance and concentration. A notable exception is encountered in patients with excessive loss of gastric juice, as in pyloric stenosis, in which the loss of chloride is greatly in excess of that of sodium, a large portion of the lost chloride being in the form of free HCl. As a rule, therefore, in the clinical study of patients, studies of salt metabolism are generally limited to the investigation of the state of chloride metabolism, for technical reasons.

Chloride is eliminated from the body chiefly in the urine and, to a lesser extent, in the sweat and feces.

Urine Chloride. Chloride is one of the substances designed by Cushny as "threshold bodies" which, being of value to the organism, are, after their passage through the glomeruli, reabsorbed into the circulation in order to maintain their normal concentration in the blood (see p. 345). The normal "renal threshold" value for sodium chloride is 560 to 570 mg. per 100 cc. of blood plasma. In the normal individual, as the plasma chloride concentration descends to this level, the elimination of chloride in the urine decreases and finally ceases entirely. Under normal conditions the quantity of chloride eliminated in the urine approximates the chloride intake; in cases in which there is marked variation in the quantity of chloride ingested it may require some time for an equilibrium to be established. For example, if the normal individual is kept upon an adequate sodium chloride intake (8-15 Gm.), the sudden addition of a large quantity of salt is usually followed by the complete elimination of the excess within forty-eight hours. However, if the individual has been maintained for prolonged periods of time on a low salt intake, the elimination of salt administered subsequently in large quantity may be delayed for several days, the body apparently retaining the added salt with greater tenacity. In conditions in which the elimination of chloride through other channels such as the skin (excessive perspiration) or gastro-intestinal tract (vomiting or diarrhea) is increased, the urine chloride content is correspondingly diminished.

Chloride in Gastro-intestinal Secretions (Fig. 5). The chloride content of the various digestive fluids varies within wide limits. The chloride content of the resting gastric juice is about 40 per cent greater than that of blood plasma, that of the intestinal secretion being about 7 per cent greater. The chloride content of hepatic bile is about equal to and that of pancreatic

juice only about 35 per cent of that of blood plasma. During the active stages of gastric secretion (digestive) the chloride concentration increases slightly, the relatively great increase in free HCl being due not so much to an increase in chloride as to a decrease in the quantity of base secreted during that period. The chloride content of the gastric juice of normal individuals may vary considerably but is relatively constant for each individual; this individual variation, however, is in no case so great as would appear to be indicated by the rather wide variation in free acid secretion. Relatively normal total chloride values may frequently be observed in cases exhibiting extremely low or absent free HCl in the gastric juice (false achlorhydria), a fact which is due either to the simultaneous secretion of excessively large amounts of base or to excessive neutralization by regurgitation of alkaline material from the duodenum.

Under conditions of normal gastro-intestinal motility very little chloride is lost in the feces. By far the greater portion of the chloride secreted into the gastro-intestinal tract is reabsorbed into the circulation. However, as will be indicated below, in the presence of diarrhea, with the passage of large quantities of fluid feces, a large amount of chloride may be lost from the body through this channel.

Chloride Elimination from the Skin. The chloride concentration of perspiration is probably approximately the same as that of blood plasma (550-650 mg. per 100 cc.), although in cases of excessive perspiration Moss found the chloride concentration to vary from 118 to 325 mg. per 100 cc. with an average of 224 mg. It is obvious that excessive perspiration may result in the loss of considerable quantities of chloride from the body, this being compensated, however, by a corresponding diminution in the amount eliminated in the urine. If excessive amounts of water are ingested during periods of active perspiration and shortly thereafter, some additional chloride may be lost in the urine with the consequent development of manifestations of salt privation. This condition was in fact observed by Moss in individuals working in deep mines, subjected to temperature above 100° F., a considerable proportion of whom developed terrific muscular cramps and intense headaches which disappeared promptly when a small amount of salt was added to the drinking water. A similar condition has been produced experimentally by McCance by means of restriction of the sodium chloride intake (with a high water intake) and the induction of profuse sweating by means of the radiant heat bath. McCance believes that the resulting symptoms of excessive fatigue, exhaustion, headache, dulness of mental processes

and cramps are dependent primarily upon depletion of body sodium, with its associated disturbance of water distribution.

DISTRIBUTION AND INTERMEDIARY METABOLISM

The factors which determine the distribution of chloride between the blood plasma and tissue fluids are the same as those mentioned as responsible for the difference in the chloride concentration of the red cells and plasma. Because of the very slight difference in the water content of tissue fluid and blood plasma the difference in the protein content of these two media is the factor chiefly responsible for the variation in their chloride content. The fact that colloids (protein) may modify the distribution of crystalloids in solution is explained on the basis of the existence of a Donnan equilibrium which may be illustrated briefly in the following manner: When two solutions (plasma and tissue fluid) are separated from one another by a membrane (capillary wall) which is impermeable for one of the ions present on one side but freely permeable for the remaining ions on both sides, an effect is exerted by the nondiffusible ion which results in the unequal distribution of the freely permeable ions on both sides of the membrane. Limiting the consideration to protein and sodium chloride and regarding the tissue fluid as being relatively protein-free, on the plasma side of the capillary wall there is a mixture of protein, sodium and chlorine ions, whereas on the tissue fluid side of the capillary wall there are only sodium and chlorine ions. Because the membrane is freely permeable for the latter two ions, an equilibrium will be established, by diffusion, which will be modified because of the presence of the nondiffusible protein ions in the plasma, the tissue fluid containing more chloride ions than the blood plasma. Although the conditions as actually existing in the living body are much more complicated than those represented in this illustration, the significance of the Donnan equilibrium in determining the distribution of electrolytes between the blood plasma and tissue fluids has been adequately supported by the investigations of many workers in this field.

Lymph from various portions of the body invariably contains more chloride than does the blood plasma, the difference being roughly proportional to the difference in their protein content. Fluid in the serous sacs of the body, which may perhaps be considered as identical with the tissue fluids and contains very little protein, has a relatively high chloride content which, in the case of cerebrospinal fluid, ranges from 720 to 750 mg. per 100 cc. expressed as sodium chloride.

Potassium is the predominant base in the cells and sodium predominates in the blood plasma and other extracellular fluids of the body (lymph, edema fluid, cerebrospinal fluid). The exact significance of this inequality of distribution and the mechanism whereby it is preserved are not clearly understood. It is clear, however, as stated by Peters and Van Slyke,²⁵ that potassium is prevented from diffusing out of the cells by a membrane or some other restraining factors present in the cellular and extracellular media. It also appears that the same or similar factors tend to prevent the undue passage of sodium into the cells under physiologic conditions. There is evidence, however, that this "impermeability" of cells, at least to potassium, and perhaps also to sodium, is by no means complete,²⁶ and it is certainly not so in a variety of abnormal conditions. Present knowledge regarding this subject may be summarized as follows:²⁷

(1) Potassium is not fixed in its predominantly intracellular position, but can move about in the body rather freely, according to the demands of shifting membrane equilibria.

(2) It probably moves in or out of cells when the usual static equilibrium is disturbed by acid-base imbalances.

(3) It moves into cells when protoplasm grows and out when protoplasm disintegrates.

(4) It passes from cells to extracellular fluids, including the plasma, when excessive quantities of water and sodium are lost from the body, as in hemorrhage, shock, adrenocortical insufficiency, intestinal obstruction and intestinal, biliary or pancreatic fistulas.

(5) It moves into the blood plasma during periods of increased muscular activity and increased metabolism, and in the opposite direction during rest or anesthesia.

(6) It appears to follow the carbohydrate cycle from muscle to liver, and back again; *i.e.*, it moves from muscle to liver with lactic acid and from liver to muscle with glucose.

(7) It often rises and falls in the plasma with the lactic acid content, as in muscular exercise, hemorrhage and asphyxia, and also frequently with the sugar content, falling after insulin and rising, temporarily, after epinephrine.

The distribution of potassium, sodium and chloride, as well as water, between intracellular and extracellular fluids of the body is influenced profoundly by the adrenal cortical hormone (cortin) (p. 238). The effect of this agent in this connection can be indicated best by enumerating the changes that occur in adrenocortical insufficiency: (a) increased urinary excretion of sodium, chloride and water; (b) decreased urinary excretion of potassium; (c) increased serum potassium concentration and

(d) decreased serum sodium and chloride concentrations, with consequent (e) hemoconcentration and (f) renal and circulatory failure and their attendant manifestations. Administration of cortin to normal subjects causes (a) retention of sodium, chloride and water, and (b) potassium diuresis.^{32,32a,33a} The mechanism of action of cortin in this connection is not known, but it appears to be exerted upon cell membranes in general and not upon the kidney alone,^{7a} maintaining their normal "permeability" or "impermeability" to sodium and potassium and thus preserving the normal distribution of these electrolytes and water between the extracellular and intracellular phases. There is some evidence that absorption of sodium and chloride from the intestine is diminished in the absence of adequate amounts of adrenal cortical hormone.

A number of steroid hormones have been found to exert an effect upon sodium, chloride and water balance. Of the adrenal cortical hormones, desoxycorticosterone causes marked retention of sodium and chloride and corticosterone moderate retention (decreased renal excretion); an opposite effect is produced by 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone (increased renal excretion). Several of the sex hormones cause retention of sodium, chloride and water, including estradiol, progesterone, estrone, pregnandiol and testosterone, in order of decreasing effectiveness in this regard. The sodium retaining effect of the estrogens may have some bearing upon the development of the condition known as "premenstrual tension."^{32a,33a}

PATHOLOGIC VARIATIONS IN BLOOD CHLORIDE AND SODIUM

HYPERCHLOREMIA AND HYPERNATREMIA

Anemia. In anemia the NaCl content of whole blood rises in proportion to the diminution in red cells, due to the relative increase in the ratio of plasma to cells. Under such circumstances the plasma NaCl concentration is not increased and the alteration in the NaCl content of whole blood is of no practical clinical significance from the standpoint of chloride metabolism.

Nephritis. Since NaCl normally is eliminated chiefly in the urine it would naturally be expected that abnormalities of renal function should be associated with alterations in the NaCl content of blood plasma and that the diminished capacity for chloride excretion which results from impaired renal function in kidney disease should be associated with a tendency toward NaCl retention in the blood. As a matter of fact, however, due

to the intervention of extrarenal factors, no constant alteration in the plasma NaCl concentration occurs in nephritis.

Nephritis cannot be conveniently classified, as was formerly supposed, into certain forms characterized by salt and water retention and others characterized by nitrogen retention. In nephrosis and nephrotic types of chronic glomerulonephritis the plasma NaCl concentration, although frequently normal or in some cases subnormal, is elevated more commonly than in other forms of nephritis. Hyperchloremia in such cases apparently bears no relation to the occurrence or degree of edema. It may perhaps be associated in some way with the diminution in plasma protein which is a constant feature of these conditions. In patients with nephrotic edema, with a previously normal plasma NaCl concentration, an increase may occur in association with diuresis and the elimination of large quantities of edema fluid. One apparent reason for the less frequent occurrence of hyperchloremia in nephrotic individuals, in spite of their failure to eliminate salt when added in large quantities to their diet, is the fact that the retained NaCl is rapidly distributed throughout the edema fluid and consequently causes little or no alteration in the chloride content of the blood plasma.

In cases of acute glomerulonephritis with edema and hypoproteinemia, in the absence of vomiting and diarrhea, the plasma NaCl may behave in a manner similar to that described above. Hyperchloremia is rarely observed in the advanced stages of chronic glomerulonephritis unless large amounts of salt are administered. Because of the diminished ability of the kidney to concentrate NaCl, if a large quantity of salt is administered without simultaneously giving an adequate amount of water, NaCl is retained in the body and hyperchloremia and hypernatremia may be observed. The level of plasma chloride in chronic nephritis bears no constant relation to the occurrence or degree of hypertension.

Urinary Obstruction. In the absence of vomiting or diarrhea hyperchloremia may be observed in cases of complete urinary obstruction due to prostatic enlargement or to urinary calculi (see hypochloremia). Because of the frequent coincident occurrence of factors which tend to produce hypochloremia, an increase in plasma Na and Cl in these conditions is not commonly observed.

Essential Hypertension. The plasma chloride concentration is within normal limits in the great majority of individuals with essential hypertension. In occasional instances minor grades of hyperchloremia are observed which, however, do not appear

(d) decreased serum sodium and chloride concentrations, with consequent (e) hemoconcentration and (f) renal and circulatory failure and their attendant manifestations. Administration of cortin to normal subjects causes (a) retention of sodium, chloride and water, and (b) potassium diuresis.^{32,32a,33a} The mechanism of action of cortin in this connection is not known, but it appears to be exerted upon cell membranes in general and not upon the kidney alone,³⁴ maintaining their normal "permeability" or "impermeability" to sodium and potassium and thus preserving the normal distribution of these electrolytes and water between the extracellular and intracellular phases. There is some evidence that absorption of sodium and chloride from the intestine is diminished in the absence of adequate amounts of adrenal cortical hormone.

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electrolyte balance have been observed during the menstrual cycle, the intermenstrual period being accompanied by decreased urinary excretion of sodium, chloride and water, with a gain in weight, while the onset of menstruation was accompanied by increased urinary excretion of these substances.³² These observations may have some bearing on the occasional occurrence of edema during certain periods of the menstrual cycle. The observation that various sex hormones exert an influence upon electrolyte balance similar to that exerted by the "salt and water hormone" of the adrenal cortex is interesting in view of the similarity of the chemical structures of these substances and because of the fact that estrus and pregnancy exert a markedly beneficial influence upon the manifestations of adrenal cortical insufficiency.

With the above exceptions, hyperchloremia and hypernatremia are seldom encountered clinically even in conditions in which NaCl retention in the body is known to exist. In no condition is hyperchloremia of such constant occurrence as to be of practical clinical significance.

HYPOCHLOREMIA AND HYPONATREMIA

Loss of Gastro-intestinal Secretions. Because of the large quantity of chloride normally secreted into the stomach, excessive vomiting, whether due to pyloric obstruction, pylorospasm, gastric irritation, upper intestinal conditions, or toxic states as in uremia and the toxemias of pregnancy, is rather consistently associated with a diminution in the level of plasma chloride. The degree of hypochloremia occurring under such conditions is dependent upon the quantity of chloride secreted into the stomach, and in conditions of true achlorhydria, as in certain cases of carcinoma of the stomach and in achylia (pernicious anemia), excessive vomiting is associated with little or no diminution in the plasma chloride concentration. If large quantities of free acid are lost in the vomitus alkalosis results. The diminution in the plasma chloride concentration with its attendant alteration in electrolyte distribution and acid-base balance is one of the most significant and important metabolic features of pyloric and upper intestinal obstruction.

Prolonged vomiting due to pyloric obstruction may be accompanied in some cases by a decrease in the serum sodium concentration. If the gastric juice contains normal or excessive amounts of free HCl, as in hypertrophic pyloric stenosis in children and stenosing duodenal ulcer, depletion of chloride is usually greatly in excess of that of sodium. The loss of chloride from the plasma results in the accumulation of bicarbonate and,

to be of clinical significance. The frequently advanced hypothesis of chloride retention or sodium retention as an important feature of essential hypertension has not been supported by recent experimental and clinical studies. In the presence of complicating nephritis or cardiac decompensation the condition of the plasma chloride may vary as indicated in the consideration of those disorders.

Cardiac Decompensation. The plasma NaCl may be normal, increased or decreased in cardiac decompensation, the presence of edema in this condition being apparently associated with a tendency toward hyperchloremia which, however, is not invariably present.

Hyperventilation. Prolonged and excessive hyperventilation, by removing excessive quantities of CO_2 from the body; results in an increase in plasma chloride due to the migration of chloride from the blood cells and tissues into the plasma (see acid-base balance, p. 272). This condition, which may be produced voluntarily, is occasionally observed clinically in hysteria and postencephalitic syndromes. Under such circumstances the diffusion of chloride into the plasma, through the neutralization of the excessive quantity of base resulting from the loss of carbonic acid, tends to prevent the development of alkalosis and tetany.

Excessive Chloride Administration. Plasma NaCl concentrations above 800 mg. per 100 cc. have been observed in patients with urinary suppression or marked oliguria following the administration, usually parenterally, of large quantities of sodium chloride solution for the purpose of inducing diuresis. This hyperchloremia is frequently accompanied by acidosis and may terminate fatally.

Glandular Effects. An increase in plasma chloride, decrease in serum potassium and a tendency toward alkalosis have been described in so-called Cushing's syndrome, frequently associated with anterior pituitary hyperfunction (basophilic adenoma).^{2,21} These changes have been interpreted as indicative of hyperfunction of the adrenal cortex, probably secondary to and dependent upon stimulation of this gland by an excess of the adrenotrophic hormone of the anterior hypophysis.

Although not exactly pertinent to the subject of increased serum sodium concentration, it should be mentioned in this connection that certain sex hormones (progesterone, testosterone, estradiol, pregnandiol) have been found to cause a marked decrease in the excretion of sodium in the urine of normal animals.²² This sodium retention is accompanied by retention of chloride and water. It is interesting, too, that changes in

the plasma chloride concentration is much below the normal threshold level (560 mg. per 100 cc.). The uremic organism likewise appears to be unable to conserve basic elements, which are eliminated in the urine and in the vomitus with the chloride ion, thus preventing the tendency toward a diminution in hydrogen ion concentration which otherwise would result from the chloride loss.

In nephritic acidosis, the decrease in plasma chloride concentration may be contributed to by the retention of other acid ions, which results in a decrease in plasma bicarbonate and passage of chloride from the plasma into the red blood cells. In this condition, the diminution in plasma NaCl is masked to some extent by the associated state of dehydration and hemoconcentration.

Decreased serum sodium and chloride concentrations are commonly encountered in severe bichloride nephrosis,¹⁸ apparently due chiefly but not entirely to loss by excessive vomiting and prolonged diarrhea.

Hypochloremia is less frequently observed than hyperchloremia in patients with edema due to nephrosis or to cardiac decompensation. However, in some such cases, due perhaps to the diffusion of chloride throughout the edema fluid, the plasma NaCl concentrations may be found to be subnormal, particularly during periods of salt restriction. Hypochloremia has been observed following frequently repeated withdrawal of large quantities of ascitic fluid in patients with hepatic cirrhosis maintained on a low chloride diet.

Diabetes Mellitus. Depletion of serum sodium and chloride may occur in patients with advanced diabetes mellitus, usually in the presence of marked acidosis. Although in some instances there is an actual diminution in the concentration of NaCl in the serum, the extent of this depletion, as in the case of nephritic acidosis, is often masked by the associated state of dehydration and hemoconcentration. It is due in part to vomiting and in part to prolonged diuresis and marked ketosis, with the excretion of large quantities of sodium in the urine, combined with ketone acids.

Infectious Diseases. Hypochloremia has been observed in several acute infections including rheumatic fever, meningitis and lobar pneumonia. It has also been reported in patients with pulmonary tuberculosis. Lobar pneumonia has for a long time been known to be associated with a diminution in the NaCl content of the urine and blood plasma, but only recently has the distribution of electrolytes in that condition been intensively investigated. It was previously believed that the decrease in

if the condition is protracted, the plasma sodium is depleted by its excessive elimination in the urine in an attempt on the part of the organism to prevent the development of marked alkalosis (p. 275). In pyloric obstruction due to carcinoma of the stomach, the gastric juice usually contains relatively small amounts of free HCl and alkalosis is less likely to occur (p. 485).

Gastric juice contains about 40 per cent more chloride (per 100 cc.) than does blood plasma. The chloride concentration of pancreatic secretion is 35-40 per cent of that of the blood plasma, that of jejunal and ileal secretion about 125 per cent and that of hepatic bile about 100 per cent. Consequently, prolonged loss of these fluids, by vomiting, diarrhea or external fistula, may result in depletion of the body chloride and hypochloremia. The occurrence and direction of a simultaneous disturbance in acid-base balance are dependent in large measure upon the hydrogen ion concentration of the secretions lost from the body and the extent of impairment of the acid-base regulating mechanism of the organism. In all of these conditions the actual extent of chloride depletion is masked by the associated state of dehydration, so that the plasma chloride concentration under such circumstances is not a true index of the chloride requirement of the body.

Marked and prolonged diarrhea, such as occurs most commonly in children, may result also in a diminished serum sodium concentration. This is due to the loss of large quantities of intestinal secretions, which contain large amounts of sodium; large quantities of bicarbonate and chloride are usually lost simultaneously, the diminution in serum bicarbonate usually exceeding that in chloride. Similar changes occur in patients with pancreatic, biliary or jejunal fistula. The relative degree of depletion of serum bicarbonate and chloride in such cases may be readily inferred from the electrolyte pattern of these fluids, as indicated in Fig. 5 (p. 227).

Renal Disease. Depletion of serum sodium and chloride frequently occurs in advanced chronic glomerulonephritis with uremia.²³ In this condition it appears to be dependent upon several factors: (a) loss of NaCl by vomiting, diarrhea and polyuria; (b) impaired capacity of the kidneys for ammonia formation, with consequent excretion of acid radicals as sodium salts rather than as ammonium salts; (c) inability on the part of the kidneys to excrete an acid urine in the terminal stages of renal failure; (d) loss of the tendency of the kidney to restrict the sodium output in the presence of serum sodium deficit.

In advanced nephritis the body appears to be unable to conserve NaCl, which is eliminated in the urine even though

capacity of the blood (increase of 2-5 volumes per cent in terminal stages). This decrease of circulating plasma volume results in (9) progressive decrease in the circulatory minute volume (blood flow), which is perhaps responsible for the progressive impairment of renal function, as evidenced by the (10) steadily increasing *NPN* concentration of the blood (100-500 per cent increase in terminal stages) and (11) the final circulatory collapse with marked fall in blood pressure. There is also (12) a gradual diminution in oxygen consumption and (13) a lowering of body temperature in the terminal stages. The loss of sodium and chloride and water in the urine is in excess of the amounts of these substances lost from the plasma, indicating that much of them must come from the tissue fluids. All of these phenomena disappear following the administration of adequate quantities of adrenal cortical hormone. The precipitation of such crises of adrenal insufficiency is favored by the administration of diets low in sodium and chloride and high in potassium, and recovery is facilitated by diets high in sodium and chloride and low in potassium.

The mechanism of production of these changes is not definitely known. It has been found that the administration of the "salt and water hormone" of the adrenal cortex to normal subjects results in decreased urinary excretion of sodium and chloride and increased urinary excretion of potassium.¹⁵ These phenomena may occur without any remarkable alteration in the concentration of sodium or potassium in the blood serum. These observations suggest that this adrenal cortical hormone may be concerned with the renal excretion of these electrolytes and that the characteristic phenomena of adrenal cortical insufficiency are dependent primarily upon increased urinary excretion of sodium, chloride and water and retention of potassium. Some believe, however, that the diminution in extracellular fluid volume (plasma and interstitial fluid) which occurs during adrenal insufficiency is due largely to passage of fluid into the cells, and that the hemoconcentration which occurs under such circumstances is due to three factors: (1) passage of extracellular fluid into the tissue cells, (2) drainage of plasma water into the extracellular fluids outside of the vascular compartment and (3) increased excretion of urine. According to this hypothesis, the most important and fundamental function of this adrenal hormone lies in the regulation of the internal distribution of body water.³⁰ There is also evidence against this hypothesis.¹² There are observations which suggest that the regulation of potassium rather than sodium metabolism is the most important fundamental action of this hormone and that the alteration

blood NaCl was due to its retention in the tissues and, possibly, particularly in the pneumonic exudate. It has been shown, however, that the chloride balance in pneumonia may be altered by varying the sodium chloride intake, the administration of small amounts of salt being associated with a negative balance and large amounts with a positive balance. This variation in chloride excretion is, however, not associated with corresponding changes in the plasma chloride concentration. It appears that in pneumonia, and perhaps in other infectious disorders, the sodium chloride concentration of the blood plasma and tissue fluids is maintained at a lower level than in normal individuals. The reason for this phenomenon is not known. It cannot be explained on the basis of what Strauss calls "historetention" nor upon the hypothesis that the primary factor is retention of sodium rather than chloride, as some observers have suggested. It would appear, as stated by Peters and Van Slyke, that sodium must either be stored in abnormally high concentration in the tissues or be retained as edema fluid. The occurrence of the characteristic crisis in pneumonia is quickly followed by restoration of normal electrolyte distribution and excretion. Similar but less marked changes may be encountered in other febrile disorders, including erysipelas, typhoid fever and pulmonary tuberculosis.

Emphysema. Hypochloremia may be observed in some cases of emphysema. It is probably due to the fact that the increased CO₂ tension of the alveolar air and blood plasma is associated with a migration of chloride from the plasma to the red cells (see acid-base balance, p. 272).

Adrenal Cortical Insufficiency.^{5,12,13,14,19} The importance of the so-called "salt and water hormone" of the adrenal cortex in the maintenance of the normal water balance and normal distribution of sodium and potassium in the body fluids has been definitely established. The following phenomena occur during periods of crisis in patients with Addison's disease and animals with acute adrenal insufficiency and are due to insufficiency of adrenal cortical function.¹³ (1) increased concentration and total elimination of sodium and chloride in the urine; (2) decreased concentration of sodium and chloride in the blood serum; (3) decreased elimination of potassium in the urine; (4) increased potassium concentration in the serum. These changes in electrolyte balance and distribution are accompanied by evidence of loss of fluid from the plasma: (5) decreased plasma volume (30-45 per cent decrease in terminal stages); (6) increased hematocrit values and red blood cell count (30-45 per cent increase terminally); (7) increased plasma protein concentration (15-20 per cent increase in terminal stages); (8) increased oxygen

changes is not understood. Slight decrease in serum sodium, as in chloride, has been observed occasionally in patients with acute hepatic necrosis²⁹ and, although rarely, in those with shock due to a variety of causes. The terminal stages of clinical and experimental hyperparathyroidism may be accompanied by a decrease in the concentration of sodium and chloride in the serum, with hemoconcentration, diminished blood flow, oliguria and renal failure.²⁸ These changes are believed to result from the prolonged diuresis which occurs in such cases. Decrease in the chloride content of the body fluids occurs in bromide intoxication, the latter ion replacing chloride. Administration of chloride hastens elimination of the bromide.

PATHOLOGIC ALTERATIONS IN URINE CHLORIDE AND SODIUM

The elimination of NaCl in the urine is decreased in most conditions associated with hypochloremia, with the notable exception of adrenal cortical insufficiency. In pyloric obstruction and other conditions associated with excessive vomiting the urinary NaCl decreases as the plasma NaCl concentration descends to the threshold value and frequently disappears at that point. Likewise, in pneumonia and other infectious disorders, during the period of activity and hypochloremia, the excretion of NaCl in the urine is greatly diminished and may be entirely lacking in some cases. With the onset of the pneumonic crisis and the restoration of the normal plasma NaCl concentration, the NaCl content of the urine increases sharply and rapidly becomes essentially normal. In advanced stages of chronic glomerulonephritis with renal insufficiency, however, NaCl may continue to be eliminated in the urine in spite of the fact that the plasma NaCl concentration may be well below the threshold value. As was indicated above, this is apparently due to an inability on the part of the organism to conserve electrolytes, this being true of base as well as chloride. In patients with edema, whether due to nephrosis, nephritis, cardiac decompensation or malnutrition, the chloride content of the urine is usually low regardless of the concentration of NaCl in the plasma. Furthermore, whereas in normal individuals the addition of sodium chloride to the diet is rapidly followed by the elimination of the excess quantity in the urine, in the presence of edema the administration of large amounts of salt is followed by its diffusion throughout the edema fluid, very little of the added amount being excreted in the urine. In some cases of severe, untreated diabetes insipidus the concentration of

in potassium metabolism mentioned above constitutes the most important and most characteristic feature of adrenal cortical insufficiency (p. 245).

Significant abnormalities in the concentrations of sodium, chloride and potassium in the serum in adrenal cortical insufficiency occur only during impending or actual crisis. Studies of the concentrations of these elements in the blood are of little or no practical value in states of mild or moderate impairment of adrenocortical function. The earliest metabolic abnormalities are reflected in excessive excretion of sodium, chloride and water in the urine, these phenomena constituting the basis for a diagnostic procedure that has proven to be of value in the early diagnosis of adrenocortical inadequacy (p. 242).

Excessive Sweating. Excessive sweating, without adequate fluid intake, may result in some degree of hemoconcentration with no change in serum sodium concentration or even an increase, because of the simultaneous loss of water with the sodium and chloride in the perspiration.²⁰ However, if excessive quantities of salt-free or salt-poor water are ingested during periods of excessive sweating, the concentration of sodium chloride in the serum may fall (p. 229), with the development of symptoms and metabolic manifestations resembling those characteristic of adrenal cortical insufficiency, including renal functional impairment with nitrogen retention in the blood. This phenomenon appears to be the basis for the production of so-called "miners'" or "stokers'" cramp, a severe localized muscle spasm, brought on particularly by exertion, and often preceded by weakness of the affected muscles.^{8,22,31} Sodium and chloride deficit has been reported in heat stroke, but negative findings have been reported by recent observers.¹⁰

Pregnancy. Normal pregnancy may be accompanied by a mild serum sodium deficit (p. 518), usually, however, without a significant decrease in serum sodium concentration. This deficit may be aggravated in pregnancy toxemias due, probably, to the influence of complicating factors, such as vomiting (p. 235).

Miscellaneous Conditions. A slight decrease in serum sodium concentration has been observed during ether anesthesia. This decrease is not accompanied by nor dependent upon increased sodium excretion in the urine and has been attributed by some to the passage of sodium from the blood and tissue fluids into the tissue cells. A slight decrease has also been observed in patients with congestive heart failure; occasionally, although less frequently, an increase in serum sodium concentration has been found in such cases. The mechanism underlying these

ABNORMAL SERUM POTASSIUM CONCENTRATION

Adrenal Cortical Insufficiency. An increase in serum potassium concentration is one of the most constant metabolic features of severe clinical (Addison's disease) and experimental adrenal cortical insufficiency, values as high as 50 mg. per 100 cc. having been reported.³⁶ As stated elsewhere (p. 238), there is considerable evidence that the fundamental action of the "salt and water hormone" of the adrenal cortex is to regulate the metabolism and distribution of potassium rather than of sodium. In experimental animals, an increase in serum potassium concentration is a more consistent feature of adrenal cortical insufficiency than is a decrease in serum sodium concentration. The increase in serum potassium is accompanied by a decrease in potassium elimination in the urine and an increase in the potassium content of the erythrocytes and muscle cells. Some believe that the adrenal cortical hormone (cortin) regulates the distribution of potassium between the tissues and the blood serum and that it may be concerned with the permeability of the cells in regard to potassium. Although the severity of the symptoms of adrenal insufficiency is not directly related to nor dependent upon the concentration of potassium in the serum and although equally high concentrations may occur in other conditions (advanced renal failure) without similar symptoms, the concentration of potassium in the serum seems to reflect the extent of adrenal cortical insufficiency. This condition, clinically and experimentally, is favorably influenced by the administration of diets low in potassium and is aggravated by a high potassium intake.

It has been found that patients and animals with adrenal insufficiency exhibit a diminished tolerance to ingested potassium.³⁶ In normal subjects, the ingestion of 10 mg. of potassium per pound of body weight is followed by no significant alteration in serum potassium concentration. Subjects with adrenal cortical insufficiency exhibit a sharp increase in serum potassium concentration (100-200 per cent), usually within thirty minutes after ingestion, with a subsequent fall of serum potassium to within normal limits at about one and one-half hours. This procedure has been proposed for the diagnosis of adrenal cortical insufficiency. As stated elsewhere (p. 244), decreased serum potassium concentration has been observed in Cushing's syndrome (pituitary basophilism), being attributed to a state of increased adrenal cortical function due to excessive secretion of the adrenotrophic hormone of the anterior hypophysis.^{2,21}

$$A = \frac{\text{urea in urine (mg. per 100 cc.)}}{\text{urea in plasma (mg. per 100 cc.)}} \times \frac{\text{Cl in plasma (mg per 100 cc.)}}{\text{Cl in urine (mg. per 100 cc.)}} \times \frac{\text{volume of largest hourly day urine (cc.)}}{\text{volume of night urine (cc.)}}$$

If the value for *A* does not exceed 25, the patient probably has Addison's disease, in the absence of renal functional impairment. If the value for *A* exceeds 30, the patient does not have Addison's disease. This procedure may be valuable as a preliminary test; if the findings are equivocal, the more drastic procedure described above should be employed.

Adrenocortical Hyperfunction. Certain patients with Cushing's syndrome exhibit changes in the serum electrolyte pattern and urinary excretion of sodium and potassium that are diametrically opposite to those in Addison's disease, suggesting a state of adrenocortical hyperfunction.^{2,21} These phenomena form the basis for a procedure that has been suggested for the demonstration of these metabolic abnormalities in early cases of this condition.⁷

The basic diet is identical with that employed in the adrenal hypofunction test (p. 242), containing 0.949 Gm. Cl, 0.592 Gm. Na and 4.062 Gm. K daily. On the first day of the study period the fluid intake is fixed at 20 cc. per kilogram of body weight, and 10 Gm. NaCl (in capsules) are given with the morning and again with the evening meal. The same regime is followed on the second day. On the third day the bladder is emptied at 8 A. M. and urine is collected for the subsequent four-hour period. On this day, 5 cc. of fluid per kilogram of body weight are given before 11 A. M. Under these conditions, the intake of Na and Cl on each of the first two days is approximately 8.6 and 12.95 Gm., respectively.

The concentration of chloride is determined in the four-hour specimen of urine obtained on the morning of the third day. Concentrations of chloride of less than 400 mg. per 100 cc. are suggestive of a state of adrenocortical hyperfunction. Values of 460-1400 mg. per 100 cc. have been obtained in subjects without suspicion of adrenocortical hyperfunction. Abnormally low values may also be obtained in patients with renal functional impairment (impaired renal concentrating ability) and possibly during pregnancy (sodium and chloride retaining effect of estrogens).

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Advanced Renal Failure. The serum potassium concentration is within normal limits in the great majority of patients with chronic glomerulonephritis. However, in the terminal stages of renal failure, with uremia, it may be markedly increased.^{23,25} The cause of this increase is not definitely known, but it may be dependent upon a combination of diminished excretion by the kidneys and the passage of excessive amounts into the blood as a result of toxic nutritional disturbances in the tissues.

Miscellaneous Conditions. Increased values for serum potassium, as high as 60 mg. per 100 cc., have been observed in clinical and experimental acute intestinal obstruction.²⁷ The possibility has been suggested that this increase may be due to increased absorption of potassium from the contents of the obstructed bowel; others attribute it to adrenal cortical insufficiency.

The injection of epinephrine is quickly followed (within one minute) by a 50 per cent increase in serum potassium concentration, which returns to normal after a few minutes.⁹ This phenomenon appears to be dependent upon mobilization of potassium from the liver. It is interesting to note in this connection that the administration of large doses of insulin, in normal subjects and in patients with diabetes, is followed by a decrease in serum potassium concentration.

An increase in serum potassium (to 32 mg. per 100 cc.) has been observed in patients with portal cirrhosis and ascites, maintained upon a diet low in sodium and chloride and high in potassium, following frequently repeated removal of large quantities of ascitic fluid.³

A decrease in serum potassium occurs during attacks of paralysis in the condition known as familial periodic paralysis. During the paralytic phase the serum potassium concentration varies considerably in different cases, being usually below 14 mg. per 100 cc. and at times as low as 8 mg. per 100 cc., although values as high as 17.4 mg. have been reported. The urine potassium excretion may decrease during an attack, suggesting that the potassium is not lost from the body but perhaps undergoes redistribution between the intra- and extracellular fluids. The relation of this phenomenon to the attacks is not understood, but the latter are relieved by administration of potassium salts.^{1,16,26,33} Values of 4.3 and 6.2 mg. potassium per 100 cc. of blood serum were reported in two cases of sprue,^{12a} the reason for this unusual finding being obscure.

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interstitial space. Consequently, an increase in the volume of extracellular fluid, constituting a state of edema, is usually accomplished entirely by expansion of the interstitial space; on the other hand, loss of plasma water as a result of dehydration is usually made up by the passage of interstitial fluid into the vascular compartment. The volume of the interstitial compartment in this way exhibits a wide range of adjustability in defense of the blood plasma volume.

TABLE 5
NORMAL QUANTITATIVE WATER TURNOVER IN 70 KG. SUBJECT
(After Adolph, E. F.: *Physiol. Rev.* 13: 336, 1933.)

Fluids excreted and reabsorbed	Volume cc.
Saliva	500- 1,500
Gastric secretion	1,000- 2,400
Intestinal secretion	700- 3,000
Pancreatic secretion	700- 1,000
Liver bile	100- 400
	3,000- 8,300
Fluids lost from the body	
Urine	600- 2,000
Feces	50- 200
Insensible perspiration	350- 700
Perspiration	50- 4,000
Milk	0- 900
	1,050- 7,800
Total fluid turnover	4,050-16,100

The quantitative turnover of water by various organs and the amount excreted under normal circumstances are presented in Table 5 and Fig. 7. When one considers that all of the secretions and excretions mentioned are derived directly from the blood plasma, it appears that a total daily fluid turnover of 4000-16,000 cc. is accomplished from a circulating medium of about 3500 cc. (plasma volume). This phenomenon is rendered possible by (1) the efficient and rapid reabsorption of the large volumes of fluid secreted into the gastro-intestinal tract (3000-8300 cc.); (2) the presence of a large and readily available reservoir represented by the extravascular fluid compartment; (3) the vast area of the capillary bed of the body (about 68,000 sq. ft.), which constitutes an enormous filtration surface for the

Chapter XII

Water Balance

A COMPREHENSIVE consideration of the subject of water balance is beyond the scope of the present discussion. However, an understanding of certain of the fundamental factors involved in the maintenance of normal water balance is essential for the

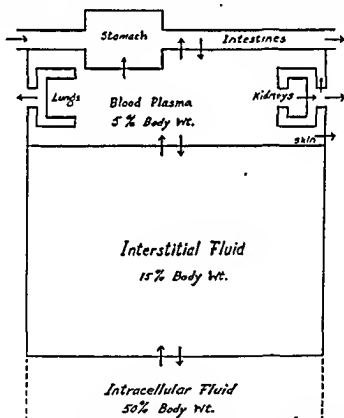


Fig. 6—Quantitative relationship between the fluid compartments of the body.
(After Gamble.)

proper understanding of certain chemical changes which may occur in the body fluids under abnormal conditions.

Water constitutes about 70 per cent of the body weight. The quantitative relationship between the great fluid compartments of the body and the chief avenues of water excretion and absorption is presented in Fig. 6 (Gamble).

As stated by Gamble, the mechanics of the circulation of the blood make the maintenance of a normal volume of fluid in the vascular compartment much more important than in the

relative to surface area, until the adult level is reached. This age difference is less marked when the blood volume is calculated on the basis of body weight. A slight increase in blood and plasma volume occurs in normal pregnancy, the increase in plasma volume being relatively greater than that in total blood volume. This disproportionate increase in plasma volume is responsible in part for the physiologic decrease in red blood cell count and plasma protein concentration during this period (p. 515).

Pathologic variations in total blood and plasma volumes may be dependent upon primary changes in the number and volume of the corpuscles. Thus, in polycythemia vera the enormous increase in red blood cells may result in a marked increase in total blood volume with a normal, increased or decreased plasma volume. On the other hand, a marked diminution in the number of red blood cells may be accompanied by a decreased or normal total blood volume and a normal, increased or decreased plasma volume. Moreover, in the presence of increased acidity of the blood, the corpuscles tend to swell, due to the imbibition of water, at the expense of the plasma volume; decreased acidity of the blood tends to produce the opposite effect.

Increased Blood Volume. An actual increase in total blood volume in relation to body weight or surface area is seldom encountered clinically. It occurs in polycythemia vera, at times in chronic leukemias before the development of anemia, occasionally during periods of water intoxication in diabetes insipidus and, similarly, in experimental water intoxication produced by excessive water administration plus the administration of pituitrin. In the last condition, the increase is due to a primary increase in plasma volume. A temporary increase in total blood volume, due to an increase in plasma volume, may occur immediately following the administration of hypertonic salt solution to patients with edema. This is due to a temporary transfer of excessively large quantities of fluid from the tissues to the blood stream; as a result of the usually prompt diuretic response, however, the plasma volume returns to normal or may actually decrease for a time if excessively large amounts of fluid are eliminated. A slight increase in blood volume has been reported occasionally in patients with essential hypertension, Raynaud's disease, thromboangiitis obliterans, hyperthyroidism, the nephrotic syndrome and congestive heart failure. However, these conditions are rarely accompanied by any significant deviation from the normal in the absence of complicating factors, the majority of which tend to lower rather than to raise the plasma volume.

Decreased Blood Volume. Diminution in blood volume may

rapid interchange of fluid between the vascular and interstitial fluid compartments.

From a practical standpoint, the investigation of abnormalities of water balance consists in study of the volume and composition of the blood and the composition of body fluids.

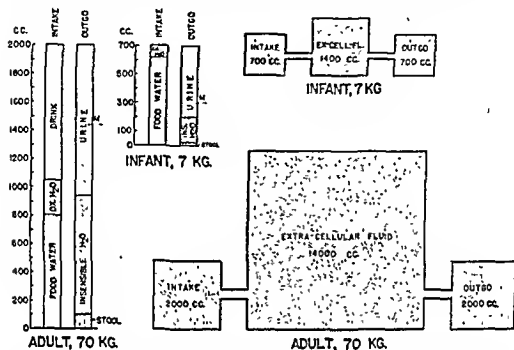


Fig. 7.—Water balance. "M" indicates the minimum water output by the kidneys compatible with maintenance of normal renal function under ordinary conditions. Insensible water (skin and lungs) is a function of the energy metabolism, serving to remove about 25 per cent of the total heat produced. (Gamble.)⁴

BLOOD VOLUME

Normal standards for blood volume vary considerably depending upon the methods employed for its determination. By the carbon monoxide method it has been found to range from 63 to 76 cc. per kilogram of body weight or 2000 to 2900 cc. per square meter of body surface. These values are somewhat lower than those obtained by the dye method which, although less accurate, is more extensively employed because of its technical availability. Employing the dye method, the normal limits for blood volume in adults have been found to be 72 to 100 cc. per kilogram of body weight or 2500 to 4000 cc. per square meter of body surface. The normal limits for plasma volume are 49 to 59 cc. per kilogram or 1400 to 2500 cc. per square meter of body surface, the cells constituting 39 to 50 per cent of the total blood volume (average about 46 per cent).¹² Calculated on the basis of surface area, the blood volume during infancy is lower than that of adults, averaging about one-third of the average adult value. Both total and plasma volumes increase gradually.

emphasized that, with the possible exception of the shock syndrome and adrenal cortical insufficiency, marked decrease in plasma volume occurs only when the factors which operate to produce it act over long periods of time. As was suggested above, any tendency toward the loss of fluid from the blood plasma is usually promptly counteracted by the passage of fluid from the interstitial fluid compartment into the vascular compartment. Since the reserve supply of fluid in the interstitial tissues is about three times as great as the normal plasma volume, it is obvious that significant diminution in the latter occurs only with extensive depletion of the body fluids. Adrenal cortical insufficiency and clinical Addison's disease constitute exceptions to this rule because of the fact that in the absence of adequate amounts of one of the cortical hormones (salt and water regulating hormone) the organism is unable to maintain the normal concentration of sodium and chloride ions in the plasma, their loss involving necessarily the simultaneous loss of considerable amounts of water (p. 238).

EXTRACELLULAR FLUID

Abnormal changes in the volume of interstitial fluid may be in the direction of either an increase or a decrease, the former constituting a state of edema and the latter a state of dehydration. The chemical composition of interstitial fluid is essentially the same as that of blood plasma, differing from the latter chiefly in that it contains much less protein, somewhat more chloride ion and less calcium and magnesium. It should be emphasized that, from the standpoint of osmotic equilibrium and acid-base balance, the most important constituents of the fluid, as of the blood, are sodium on the base side and chloride and bicarbonate on the acid side. Moreover, as stated by Gamble^{3,4} the integrity of the chemical pattern of extracellular fluid (blood plasma and interstitial fluid) and the stability of its physicochemical properties depend largely upon the maintenance of normal quantities of sodium and chloride. Changes in bicarbonate, except those due to its excessive administration, are usually dependent upon primary changes in the amount of chloride ion or upon the presence of increased quantities of abnormal acids, such as the ketone acids. The relation of these elements to the hydrogen ion concentration and osmotic value of extracellular fluid may be summarized as follows:^{3,4}

(1) The two factors which immediately control the pH of extracellular fluid are the relative concentrations of free carbonic acid and of bicarbonate, the normal ratio being approximately 3:60.

be due to a decrease in the volume of corpuscles or of plasma or both. Immediately after a severe hemorrhage the blood volume is decreased due to loss of both cells and plasma. The plasma volume is quickly restored, particularly if fluids are administered in adequate amount and, after the first twenty-four hours, the plasma volume may be actually greater than normal. However, the total blood volume is not restored for some time, depending upon the severity of the blood loss. This restored plasma is usually deficient in protein, the plasma protein concentration usually being restored to normal before the red blood cells and hemoglobin concentration. In the majority of chronic anemias the total blood volume is usually normal or slightly subnormal, the plasma volume being normal or increased. In pernicious anemia the total blood volume is low, the plasma volume being usually normal or subnormal. A decrease in total blood volume has also been observed in hemolytic jaundice, the plasma volume usually being somewhat increased. Strangely enough, elevated blood and plasma volumes have been reported in patients with splenic anemia and portal cirrhosis. The data available in such cases are not sufficient to permit any definite statement regarding the usual state of blood and plasma volume in these conditions.

A marked decrease in total blood volume is a prominent feature of the shock syndrome, which may be due to a variety of causes. Except in the presence of hemorrhage, this decrease is usually due almost entirely to a decrease in plasma volume, contributed to largely by the transudation of fluid, and usually also some protein, through the abnormally permeable capillaries. This results in a state of hemoconcentration, as evidenced by high hematocrit values and red blood cell counts. Subnormal total blood and plasma volumes, with hemoconcentration, may also be observed under the following conditions: (a) prolonged water restriction; (b) excessive water loss (diuresis, diarrhea, vomiting, intestinal fistula, pancreatic and biliary fistula, sweating); (c) acidosis, in which the loss of plasma water may be due in part to diuresis and in part to transfer of water from the plasma to the cells; (d) advanced diabetes mellitus, in which the low plasma volume may be due in part to diuresis and in part to acidosis; (e) excessively high external temperatures, in which large quantities of water may be lost through the skin and lungs; (f) occasionally in uremia, due to such complicating factors as acidosis, vomiting, diuresis, diarrhea, etc.; (g) ether anesthesia; (h) adrenal cortical insufficiency, in which the low plasma volume probably results from the excessive loss of sodium and chloride ions, with water, in the urine, or their passage from the plasma into the tissues (p. 238). It should be

it follows that the total base concentration determines the total osmotic value of the electrolytes.

(7) Because sodium constitutes such a large portion of the base structure of extracellular fluid, the osmotic value of the latter rests almost entirely on this element.

Decreased Interstitial Fluid (Dehydration). From a biochemical standpoint a discussion of dehydration, a decrease in interstitial fluid, resolves itself into a discussion of its consequences, namely, the resulting changes in the electrolyte composition and acid-base balance of the blood plasma. Changes in the blood plasma may be accepted as indicative of changes in the interstitial fluid under these circumstances. The nature of such changes in some of the common clinical disorders associated with dehydration is presented in Fig. 8.

Changes in the volume and structure (electrolyte pattern) of extracellular fluids go hand in hand.^{1,2} In the great majority of instances, dehydration, as encountered clinically, is due to excessive loss of one or more of the body secretions or excretions (vomiting, diarrhea, intestinal obstruction, intestinal fistula, pancreatic fistula, excessive diuresis or sweating). The effects of such loss upon the electrolyte pattern of the blood plasma and other extracellular fluids depend largely upon the chemical structure of the fluid which is lost and upon consequent abnormalities in renal function.

Considerable light has been thrown upon the important relationship between the sodium content of extracellular fluid and the distribution of water in the body as a result of studies of the action of the adrenal cortical hormone (p. 238). In the absence of adequate amounts of this factor, there is excessive excretion of sodium in the urine and a diminution in its concentration in the blood serum. The loss of sodium from the body is accompanied by a simultaneous loss of chloride and water, with consequent dehydration and hemoconcentration. However, there is experimental evidence that the production of these latter phenomena is not necessarily dependent upon increased loss of these elements in the urine, but that they may result from changes in the distribution of sodium, and consequently water, between the extracellular and intracellular fluids.^{5,6,13} In any case, the shift in water appears to be determined by the movement of sodium, illustrating the importance of the latter in connection with all problems of water balance and distribution.

Sodium chloride depletion has been produced in human subjects⁹ by means of a NaCl-free diet, ingestion of large quantities of distilled water and the induction of profuse sweating through the medium of radiant heat baths. The consequent

(2) Since the normal concentration of the former is maintained quite accurately by the respiratory mechanism, deviations from the normal pH (acidosis or alkalosis) are almost invariably caused clinically by changes in the concentration of bicarbonate ion.

(3) Changes in the latter are never primary, but always result from alteration in some other part of the electrolyte structure. For example, if the sum of the other acid radicles (Cl , PO_4 , SO_4 , organic acids) is increased, an equivalent amount of bicarbonate will be dispossessed of base and liberated as free carbonic acid (acidosis). On the other hand, if the sum of the other acid radicles is decreased, the base previously combined with these radicles is immediately covered by bicarbonate ion,

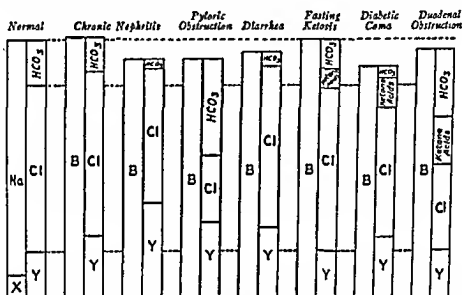


Fig. 8.—Changes in the electrolyte pattern of the plasma in various disease conditions. Dotted lines indicate the normal levels of bicarbonate and chloride. B = total base; Y = unnamed acid radicles. (After Gamble.)

which is then increased correspondingly (alkalosis). In other words, the value for bicarbonate in the plasma is determined by the extent to which fixed base exceeds the sum of the acid radicles other than bicarbonate.

(4) Obviously, therefore, the normal value for bicarbonate depends upon the integrity of the entire electrolyte structure, the most important components of which, from a quantitative standpoint, are sodium and chloride (Fig. 8).

(5) The osmotic value of extracellular fluid is, of course, determined by the total concentration of its chemical components, but chiefly that of the electrolytes.

(6) Since changes in the concentration of chloride and other acid ions (other than bicarbonate) are offset by reciprocal changes in the concentration of bicarbonate, as stated above,

the plasma and other extracellular fluids (pp. 388, 392), and may play an important part in contributing to the serious consequences of severe dehydration.

As indicated in Fig. 8, ketosis may occur in the presence of alkalosis as well as acidosis. For example, in intestinal obstruction, ketosis may develop as a result of carbohydrate starvation, but the acidifying effect of the ketone acids is usually insufficient to counterbalance the alkalinizing effect of the excessive accumulation of bicarbonate, which results directly from the depletion of chloride ion. From the standpoint of therapy, it is of the utmost importance to recognize that in states of dehydration, changes in the chemical structure of the body fluids occur simultaneously with changes in their volume. It is important to recognize also that, as emphasized by Gamble, both defects (volume and structural) are reparable by the same agents, water, sodium and chloride, regardless of whether the acid-base balance is disturbed in the direction of acidosis or alkalosis. The only defect of structure that cannot be repaired by this means is the presence of excessive quantities of ketone acids, which may be corrected by the administration of glucose. When adequate amounts of water and sodium chloride are administered, the correct degree of retention and excretion of the sodium and chloride ions necessary to reestablish the normal acid-base balance depends upon the accuracy of renal function.

Increased Interstitial Fluid (Edema). Edema consists in the abnormal accumulation of interstitial fluid, either local or general. A detailed description of the factors involved in the pathogenesis of edema is beyond the scope of the present discussion. However, since the chemical composition of edema fluid is determined to some degree by the nature of the mechanism underlying its production, a brief consideration of these factors seems desirable.

The normal interchange of fluid between the vascular compartment and the tissue space depends primarily upon four factors: (1) the capillary blood pressure (13-35 mm. Hg), lower at the venous end than at the arterial end of the capillary, which tends to drive fluid toward the tissue spaces; (2) the colloid osmotic pressure (oncotic pressure) of the blood plasma (about 25 mm. Hg), dependent chiefly upon the concentration of plasma albumin (p. 77), which tends to draw fluid into the vascular compartment, counteracting the effect of the capillary blood pressure, (3) the relative impermeability of the capillary wall to protein; (4) the lymphatic circulation, which aids in the removal of fluid from the tissue spaces. A fifth factor is perhaps operative, namely, the resistance offered by the tissues them-

subjective and objective manifestations were similar to those occurring as a result of sodium deficiency produced by other methods. The chief objective phenomena were (1) lowered plasma volume, (2) decreased concentration of sodium and chloride, (3) hemoconcentration (increased erythrocyte count and hemoglobin and protein concentrations) and (4) impaired renal excretion of nitrogenous substances, as evidenced by increased blood urea nitrogen concentration. An increase in blood nonprotein nitrogen is seen frequently under such circumstances^{2,7,10} (excessive sweating, pyloric obstruction, prolonged diarrhea, adrenal cortical insufficiency, diabetic acidosis, extensive burns, intestinal fistula, and so on). Certain observations suggest that dehydration per se may have a deleterious effect on renal function.² Some believe that it is also accompanied by increased protein catabolism, which may contribute to the increase in blood NPN.¹⁰ Severe dehydration, with hemoconcentration due to diminution in plasma volume, probably results in impaired circulation through the kidneys (increased blood viscosity) which, together with the decreased volume of plasma water, leads to a decrease in glomerular filtration. This phenomenon may be further aggravated by the frequently associated fall in arterial blood pressure (shock) and the increased concentration of plasma proteins (hemoconcentration), both of which tend to diminish glomerular filtration.

Among the common causes of clinical states of dehydration are those conditions characterized by excessive loss of gastrointestinal secretions or by excessive diuresis. As stated previously, the changes in acid-base balance that may occur as a result of the loss of any of these fluids depend primarily upon the relative amounts of sodium and chloride ions which they contain. If sodium is lost in excess of chloride (pancreatic, ileal, jejunal secretion, urine) there is a tendency toward a decrease in the pH of extracellular fluid (acidosis); if chloride is lost in excess of sodium (gastric juice) the pH tends to increase (alkalosis). This tendency toward alteration in the chemical structure of the extracellular fluids is influenced by the efficiency of renal function, the kidneys being the chief organ of regulation of the composition of extracellular fluids. As stated by Gamble, in their defense of the chemical pattern of these fluids, the kidneys are required to produce a solution of substances (urine) widely differing from the blood plasma from which it is formed. The extent of this difference is indicated in Fig. 13, page 343, which illustrates the chemical composition of the plasma and urine under average normal conditions. Obviously, renal functional impairment results in dislocation of the chemical structure of

yields information of diagnostic importance. The chemical constituents of edema fluids are derived chiefly from the blood plasma, the composition of such fluids being dependent to some extent upon the permeability of the capillaries in the region involved, a factor which varies considerably in normal individuals in different vascular areas, and pathologically in response to abnormal stimuli. Alterations in the chemical composition of cerebrospinal fluid are considered elsewhere (p. 502). No attempt will be made to discuss in detail the chemical composition of edema fluid, attention being directed particularly to alterations which are of significance from the standpoint of diagnosis.

Specific Gravity. Crystalloids, being relatively readily diffusible, exist in practically the same concentration in edema fluids in different situations formed under similar conditions; such fluids, however, vary in specific gravity due to differences in their protein content, which are dependent upon variations in the permeability of the capillaries in different portions of the body. Starling showed that normally the lymph coming from the lower extremities contains 2-3 per cent of protein, that coming from the intestines 4-6 per cent, while that from the liver contains 6-8 per cent. Similarly, it has been found that, in general, the quantity of protein in edema fluid in different localities varies in decreasing order as follows: (1) pleura, (2) peritoneum, (3) cerebrospinal, (4) subcutaneous. Some observers would vary this order slightly, reversing the positions of peritoneal and pleural effusions and of subcutaneous and cerebrospinal fluid. The statement is frequently made that the specific gravity of transudates is usually below 1.015 and that of exudates above 1.018; however, in view of the fact that the crystalloid content, with the exception of minor differences due to the existence of a Donnan equilibrium (p. 230), varies but slightly, the specific gravity varying more or less in direct proportion to the concentration of protein, there must be many exceptions to this rule since under identical conditions the protein concentration of edema fluids in various situations differs, as stated above. However, with these reservations in mind, statements made with regard to the difference in specific gravity of transudates and exudates may be accepted as a fairly satisfactory working basis for distinguishing between these two types of edema fluid. Values as high as 1.035 may be observed in inflammatory pleural and peritoneal exudates and values as low as 1.005 in subcutaneous transudate fluid.

Protein. Under normal conditions the fluid in the tissue spaces and in the serous cavities may be regarded as essentially

selves to the accumulation of fluid, usually termed the tissue tension, which varies in different parts of the body. Regardless of the nature of the clinical disorder in which edema may occur, the development of this phenomenon is almost invariably dependent upon abnormality in one or more of these fundamental factors. The various mechanisms responsible for the development of edema may therefore be outlined as follows (Landis):

- (1) Increased Capillary Blood Pressure.
 - (a) Congestive heart failure.
 - (b) Thrombophlebitis.
 - (c) External pressure on veins.
 - (d) Heat.
 - (e) Dependency.
 - (f) Vasodilatation (hemiplegia, trophedema).
- (2) Decreased Colloid Osmotic Pressure.
 - (a) Loss of albumin.
 - (1) Urine.
 - (2) Ascites, etc.
 - (b) Inadequate protein intake.
 - (1) Dietary restriction.
 - (2) Impaired absorption (vomiting, diarrhea, mucosal edema, etc.).
 - (c) Impaired synthesis of plasma protein.
 - (1) Chronic infection.
 - (2) Anemia.
 - (3) Cachexia.
 - (4) Hepatic dysfunction.
 - (5) Nephritis.
 - (d) Sudden plasma dilution.
 - (1) Following sudden recovery from dehydration (diabetic coma, diarrhea in children, etc.).
 - (2) Following acute massive hemorrhage.
- (3)
 - (a) Inflammation (infection, burns, etc.).
 - (b) Acute glomerulonephritis.
 - (c) Anemia.
 - (d) Congestive heart failure (anoxemia).
- (4) Decreased Lymphatic Drainage.
 - (a) Lymphedema.
 - (b) Increased venous pressure (congestive heart failure).

Among the important contributing factors the following may be mentioned: (a) a high intake of sodium if water is available, and vice versa; (b) low tissue tension, as in the case of the eyelids and genitalia, which favors the accumulation of fluid in those situations. Except under unusual circumstances, these contributory factors serve merely to increase the tendency to edema in the presence of one or more of the fundamental factors enumerated above.

TRANSUDATES AND EXUDATES

The chemical examination of abnormal accumulations of fluid in the subcutaneous tissues and serous cavities frequently

upon other forces which are believed to be responsible for the production of edema in chronic nephritis and myocardial failure. The protein concentration of blister fluid is also very high. Of particular interest is the observation that the fluid of angioneurotic edema contains large amounts of protein, the inference being that this condition is associated with a marked increase in capillary permeability.

Albumin usually constitutes by far the largest part of the protein present in edema fluid, globulin occurring in smaller amounts and fibrinogen being seldom observed except in acute inflammatory exudates. As suggested by Oswald, the ability of these proteins to pass through the capillary wall is dependent upon the size of their molecules, those of greater size being more viscous and therefore passing with greater difficulty. The viscosity of plasma proteins varies in the following increasing order: albumin, pseudoglobulin, euglobulin and fibrinogen; therefore, in transudates, albumin may be present alone or may be accompanied by a small amount of pseudoglobulin; euglobulin and fibrinogen are usually present only in inflammatory exudates, the last named being indicative of an intense inflammatory reaction. Pneumococcus exudates appear to be particularly rich in fibrinogen, which is at times present in such high concentration that the fluid may coagulate spontaneously.

In some cases a protein substance closely resembling mucin has been found in inflammatory exudates. Whereas the proteins mentioned above are believed to be derived from the blood plasma, this substance (mucin) appears to be a product of the inflamed cells. It has frequently been observed in joint fluids which, whether transudates or exudates, usually contain larger quantities of protein than similar fluids in other situations, the concentration of mucin being quite constant and apparently independent of the nature of the pathologic process.

Glucose. With the exception of cerebrospinal fluid, the fluid of the tissues and that of the pleura, peritoneum and other serous cavities contains sugar in practically the same concentration as that of the blood. The sugar present in these fluids appears to be chiefly glucose. Alterations in the glucose concentration of the blood are reflected in parallel changes in the glucose content of the various tissue fluids. The glucose content of pleural and peritoneal transudates is usually practically the same as that of the blood. The glucose content of inflammatory exudates is relatively low due to the destruction of glucose by the action of bacteria and cells present in the fluid, the degree of this reduction being dependent somewhat upon the intensity of the inflammatory process. The significance of this observation in the differen-

protein-free filtrates of the blood plasma. The cerebrospinal fluid, which is the only one normally present in amounts large enough to enable quantitative analysis to be made with any degree of accuracy, normally contains 15-45 mg. of protein per 100 cc. If these figures may be accepted as representative of tissue fluids throughout the body it is evident that their normal protein content is practically negligible as compared with that of blood plasma (6-8 Gm. per 100 cc.).

The extremely low protein content of these fluids is due to the relatively poor diffusibility of protein through normal capillaries. Since inflammatory processes are associated with a marked increase in the permeability of the capillaries in the involved area, the degree of such increase being dependent upon the intensity of the process, the protein content of inflammatory exudates is relatively high. In purulent exudates resulting from a severe inflammatory process, as illustrated by empyema, the protein content of the serous portion of the fluid, obtained by centrifugation, may be approximately the same as that of the blood plasma. In the case of exudates resulting from inflammatory processes of lesser intensity, such as tuberculous pleurisy, tuberculous peritonitis, pneumonic pleurisy, meningitis, etc., the total protein concentration usually ranges from 0.1 to 5 Gm. per 100 cc., being usually lower in the cerebrospinal fluid than in the peritoneal and pleural exudates. In contradistinction to the relatively high values observed in inflammatory fluids, the protein content of noninflammatory edema fluids or transudates is relatively extremely low since their pathogenesis is usually independent of alterations in capillary permeability. Thus the protein content of subcutaneous edema fluid is frequently below 0.1 Gm. per 100 cc., that of pleural and peritoneal transudates occurring as a result of myocardial failure, nephrosis, uncomplicated cirrhosis of the liver, or the like being correspondingly low (0.1-1.0 Gm. per 100 cc.). It must be realized however, that, particularly in the case of pleural and peritoneal transudates, if the effusion has existed for some time, water may be reabsorbed more rapidly than solids, resulting in a slowly increasing concentration of protein which may eventually approach that of true exudates. It has been estimated that in the presence of a normal serum protein concentration, the presence of increased capillary permeability can be assumed with some degree of certainty only if the protein content of the edema fluid exceeds 4.1 Gm. per 100 cc.¹¹

A relatively high protein content has frequently been found in the subcutaneous edema fluid of acute nephritis, suggesting its dependence upon generalized capillary injury rather than

pseudochylous, depending upon their fat content and their pathogenesis. True chylous effusions are due to the escape of chyle from a ruptured or obstructed thoracic duct into the pleural or peritoneal spaces. The fat content of chylous fluid naturally varies with the quantity of fat ingested, being modified to a certain extent by processes of effusion or resorption occurring in the pleura or peritoneum. Values of from 0.05 to 3.85 Gm. per 100 cc. have been reported. The concentration of cholesterol and lecithin, although increased, is usually low in proportion to that of neutral fat and fatty acids. Such fluids usually contain relatively high concentrations of protein, this factor also being largely dependent upon the composition of the diet, and spontaneous coagulation occurs not infrequently.

The term "chyliform effusion" is applied to fluids which may be identical in appearance with those described above, the fat content of which, however, is due not to chyle but to fatty degeneration of the cells present in the effusion or of those lining the walls of the cavity involved. Differentiation between chylous and chyliform effusions is frequently difficult since in both instances the turbidity is probably due to emulsified fat. However, in some cases the cholesterol and lecithin content of chyliform effusions is relatively higher and the fat content relatively lower than those of true chylous fluids. The term "pseudochylous effusion" is applied to fluids which may be turbid or milky in appearance but contain little or no fat, the turbidity being due chiefly to lecithin and cholesterol. It has also been demonstrated that albumin in a highly dispersed state may impart a milky appearance to such fluids. Subcutaneous pseudochylous fluid in the lower extremities and the scrotum may result from obstruction of lymphatic vessels by filaria. Similar fluids in the pleural and peritoneal cavities have been observed in lipoid nephrosis and in chronic glomerulonephritis with superimposed nephrotic lesions. They have also been observed in association with carcinoma of the peritoneum and in tuberculous pleurisy and peritonitis. Pseudochylous fluids may appear to be relatively clear when first removed, the turbidity and milky appearance increasing upon cooling. Relatively large amounts of protein may be present, varying from 0.1 to 4.2 Gm. per 100 cc., and spontaneous coagulation, though not as commonly observed as in true chylous effusions, may nevertheless occur.

Other Constituents. Creatinine, uric acid and particularly urea are present in exudates and transudates in practically the same concentration as in the blood. Some observers have found that in nephritis edema fluid may at times contain more non-protein nitrogen than the blood, an observation which may be of

tial diagnosis of meningeal and cerebrospinal lesions is discussed in the section on cerebrospinal fluid (p. 505).

Chloride. The factors which determine the relationship between the chloride content of blood plasma and tissue fluid are discussed elsewhere (p. 230). The chloride content of non-inflammatory edema fluid, or transudates, is higher than that of the blood plasma, ranging from 720 to 750 mg. per 100 cc., the difference being due to the existence of a Donnan equilibrium dependent upon the higher concentration of protein in the plasma as compared with normal tissue fluid and transudates. The chloride content of inflammatory exudates, which are relatively rich in protein, is lowered, approaching that of blood plasma, the degree of diminution varying roughly directly with the increase in the concentration of protein in accordance with the laws governing the concentrations of readily diffusible substances on two sides of a semipermeable membrane under such circumstances (see p. 230). The chloride content of pleural effusions in pneumococcus pneumonia is particularly low because of the low chloride concentration of the blood plasma.

Lipid. Neutral fat and fatty acids are not usually present in transudates or inflammatory exudates. A small amount of lecithin, varying from 20 to 100 mg. per 100 cc., may practically always be demonstrated, existing partly in the free state and partly in combination with protein. Cholesterol does not appear to be a constant constituent of transudate fluid but is practically always present in inflammatory exudates, particularly those of long standing, being probably derived in part from degenerative changes either in the cells present in the fluid or in those lining the serous sac or the abscess cavity. The cholesterol content of such long-standing effusions may decrease markedly following repeated tapping, values ranging from 1 to 4.5 Gm. per 100 cc. having been observed to fall to 20 to 50 mg. following repeated aspiration. In some cases exhibiting high cholesterol values fat is also present, having been observed particularly in tuberculous pleural and peritoneal effusions. Capillaries appear to be permeable to cholesterol to about the same extent as to protein, this fact accounting for the very low cholesterol content of transudate fluids. This is true usually even in cases of the nephrotic syndrome, in which the concentration of cholesterol in the blood plasma may be enormously increased. In inflammatory exudates, associated with increased capillary permeability, the lipid content of the edema fluid roughly parallels its protein content.

Effusions which contain lipids in sufficient quantity to cause a milky appearance are designated chylous, chyliform and

Chapter XIII

Acid-base Balance

HYDROGEN ION CONCENTRATION .

THE term "hydrogen ion concentration," though introduced into chemistry a number of years ago, has only in recent years become familiar in the literature of the physician.

One of the first difficulties with a subject like hydrogen ion concentration is the appreciation of the unit of measurement. In anatomy the doctor can see for himself the gross anatomy with the naked eye. Even in histology and bacteriology we soon accustom ourselves to think in terms of microscopic measurements. With the introduction of not only molecular and atomic concepts, but ions, there is danger of failing to realize how great is the difference in scale. The step from gross structure to cellular is short indeed compared with the great transition to the realm of molecules, atoms, and ions.*

We should remember that it is necessary to change the unit of scale or measurement with the size of the object to be measured. The method of titration with indicators which give one color in an acid solution and another in alkaline solution is satisfactory for determining total acidity, but it does not enable us in all cases to determine the true or momentary acidity of a liquid, that is, its hydrogen ion concentration. For instance, while acetic acid and nitric acid of the same strength will require about the same amount of base to neutralize them, their influence on living tissue will be governed by the proportion of hydrogen which has assumed a positive charge, and this is termed the hydrogen ion concentration, that is, the specific or momentary acidity.

In the case of acetic acid its taste† is enjoyed in 4 per cent strength as is apparent by the popularity of vinegar, but no one

* An *ion* is an electrified atom or group of atoms, and as electricity always manifests itself in two forms, positive and negative, there will always be a positive and negative ion in more or less intimate relation. (Ions must not be confused with *electrons* which are particles of negative electricity constituting parts of atoms.)

Hydrogen ion concentration relates to the condition assumed by acids and alkalis when in solution

† Dr. Henry Leffmann's explanation of ionization and taste:

"The much higher ionized nitric acid causes a greater reaction to the terminals of the lingual branch of the fifth nerve as well as its very irritating effect on all living tissue. Example: Strawberry juice tastes sourer than tomato juice because it has a

significance but the practical importance of which has not been demonstrated. The calcium content of transudates, ranging from 4.5 to 5.5 mg. per 100 cc. in the case of fluid with a low protein content, apparently represents the normal diffusible fraction of serum calcium. With increasing values for protein in both transudates and exudates the calcium concentration increases, the increase representing a nondiffusible fraction, which is probably in combination with protein. Transudates contain approximately the same concentration of inorganic phosphorus as the blood serum, and the concentration of bicarbonate is somewhat higher and that of sodium somewhat lower than in serum. The concentration of magnesium in transudate fluids averages about 65 per cent and that of potassium about 80 per cent of that in the serum. In the presence of increased protein concentration in the fluid, the concentration of magnesium increases.

Small amounts of bilirubin may be present in transudate fluid in the presence of hyperbilirubinemia. Larger amounts may be found in exudate fluids under such circumstances in concentrations varying roughly in proportion to the concentration of protein in the fluid. Bilirubin has been demonstrated in pleural and peritoneal effusions in patients with congestive heart failure and cirrhosis of the liver without hyperbilirubinemia.

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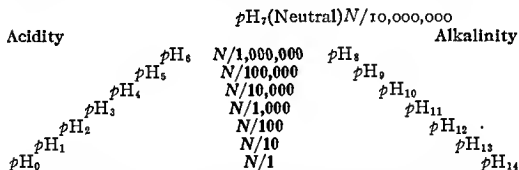
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liters, it can only take place by some increase of an opposing (negative) ionized atom or group. This is usually the group OH, the characteristic and active group of alkalis. Therefore, in accordance with the above rule, this increase of negative ion diminishing the effective activity of the positive ion represents a greater dilution of the latter and hence a higher minus figure. But the minus sign being omitted as usual, the figure stands as, for example, pH_8 which marks a diminution of the hydrogen ion concentration by ten times that of pH_7 . Proceeding in this way by adding substances containing hydroxyl ions, of which the common alkalis are the best representatives, we gradually reach a point at which the alkalinity is equivalent to and will exactly neutralize the hydrogen ion concentration of pH_0 . This alkalinity is represented by the figure pH_{14} .

The following graph shows this:



Practically all the body fluids are represented by large unwieldy figures near the apex of this graph and so a short way of writing them is to use the negative power of ten to express the denominator. The abbreviation pH stands for the power (logarithm) of the number expressing the concentration of hydrogen ions. For example, the hydrogen ion concentration of normal blood is about .000,000,04 N , which in its abbreviated form is $pH\ 7.39$.

The blood plasma is normally slightly alkaline in reaction, the hydrogen-ion concentration ranging from $pH\ 7.3$ to $pH\ 7.5$, averaging 7.35, venous blood ($pH\ 7.32$) being slightly more acid than arterial blood. Physiologic processes are very sensitive to even minute changes in the reaction of the body fluids and, even in disease, variations beyond the limits of $pH\ 7$ to $pH\ 7.8$ are almost never observed. A remarkably efficient mechanism prevents the sudden variations in hydrogen ion concentration which would in its absence occur in the blood and tissue fluids as a result of either the introduction of acid and basic substances from without or their elaboration in the tissues in the course of metabolic activity. This mechanism may be conveniently considered under two headings: (1) chemical and physicochem-

would sanely think of drinking 4 per cent nitric acid, for it would have a markedly irritating if not toxicologic effect. Yet both these acids give about the same reading when titrated. Obviously, then, since acids of the same strength differ so greatly in their action on living tissue, it becomes a matter of necessity for the physician to appreciate this new scale of acidity and alkalinity, for it affords an insight into the why and how of the physiologic actions of the acid and alkaline fluids of the body.

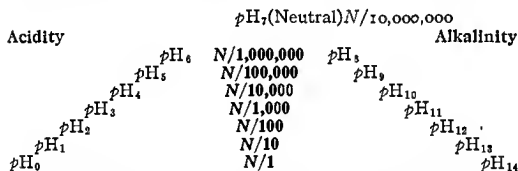
This difference in action is due to the proportion of acid which acquires activity by reason of the electrical charge assumed by the hydrogen in the molecule. Thus when the two acids are diluted with water so as to give the 4 per cent solution a certain proportion of each acid undergoes a change by which the hydrogen becomes positively charged and the rest of the molecule negatively charged. The extent to which this change takes place determines the action on living tissue. This change is termed "ionization," and each chemical compound has a special susceptibility to such change when brought into solution. In the case under consideration nitric acid in a given dilution will suffer ionization to about 80 per cent, while acetic acid in an equivalent dilution will be ionized to less than 1 per cent. These differences account for the use of the term "strong acid" (nitric) and "weak acid" (acetic). This difference in the electric state of the hydrogen atom constitutes the "hydrogen ion concentration." It is expressed by the symbol pH , with an attached number to indicate the proportion. This number is based on the fact that pure water under normal conditions ionizes itself to the extent of producing 1 Gm. of ionized hydrogen in 10,000,000 liters; in other words, $1/10,000,000$ of the liquid is ionized. Any numerical expression of this fact will be the expression of the hydrogen ion concentration (H ion conc.). The method adopted is to take the logarithm of this fraction. The logarithm of $1/10,000,000$ is minus seven (-7). If the solution is ten times as strong the logarithm would be -6 , and so on down to any strength. In practice, however, the minus sign is omitted, with the somewhat awkward result that as the amount of ionization increases the figure decreases, hence pH_1 represents a very much more strongly ionized solution than pH_7 . Further, if the ionization of the hydrogen falls below 1 Gm. in 10,000,000

greater H-ion concentration, but titration with a base shows tomato juice has greater total acidity.

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liters, it can only take place by some increase of an opposing (negative) ionized atom or group. This is usually the group OH, the characteristic and active group of alkalis. Therefore, in accordance with the above rule, this increase of negative ion diminishing the effective activity of the positive ion represents a greater dilution of the latter and hence a higher minus figure. But the minus sign being omitted as usual, the figure stands as, for example, pH_8 which marks a diminution of the hydrogen ion concentration by ten times that of pH_7 . Proceeding in this way by adding substances containing hydroxyl ions, of which the common alkalis are the best representatives, we gradually reach a point at which the alkalinity is equivalent to and will exactly neutralize the hydrogen ion concentration of pH_0 . This alkalinity is represented by the figure pH_{14} .

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ical reactions in the blood and tissues; (2) excretory processes by means of which the excess of acid or alkali is eliminated from the body.

BUFFER REACTIONS IN THE BLOOD AND TISSUES

Under normal conditions the hydrogen ion concentration of blood plasma is not materially affected by the addition of relatively large quantities of acid or basic substances. This constancy of reaction is maintained through the operation of certain substances which "soak up" the excess of hydrogen or hydroxyl ions and which are designated "buffer substances" after the term "puffer (tampon) substances" suggested by Sørensen. These buffer systems may be considered under three headings:

- (a) The bicarbonate system.
- (b) The phosphate system.
- (c) Plasma proteins, hemoglobin and chloride.

THE BICARBONATE SYSTEM.

The bicarbonate system consists of a mixture of carbonic acid (H_2CO_3) and bicarbonate (BHCO_3), the most important of the basic elements (B) in this connection being sodium (Na) and potassium (K). The buffer efficiency of this system depends upon the laws governing the reactions of mixtures of weak acids and their alkaline salts. Carbonic acid acts as a very weak acid, producing relatively few hydrogen ions, and sodium bicarbonate acts as a weak base, producing but few hydroxyl ions, the hydrogen ion concentration of mixtures of these two substances being proportional to the relative quantity of each present in the mixture. In other words $\text{cH}^+ = \frac{\text{H}_2\text{CO}_3}{\text{BHCO}_3}$, increasing or decreasing in direct proportion to increase or decrease in this ratio.

Acids stronger than carbonic acid cannot exist as such in the presence of bicarbonate, reacting with the latter to form neutral salts and carbonic acid.



In this way the addition of a strong acid results in the formation of carbonic acid which is a weak acid, dissociating to a relatively slight degree and giving rise to but few hydrogen ions with very little consequent alteration in the hydrogen ion concentration of the mixture. In a similar manner the addition of a strongly alkaline substance to a carbonic acid-bicarbonate mixture re-

$$^* \text{cH} = \frac{1}{10^{\text{pH}}} \text{ or } 10^{-\text{pH}}$$

sults in the formation of bicarbonate which is weakly alkaline and gives rise to but few OH ions according to the following equation:

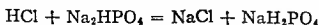


The bicarbonate system is particularly effective in maintaining the normal hydrogen ion concentration of the blood plasma inasmuch as the excess of carbonic acid formed as a result of the interaction of bicarbonate with relatively strong acids formed during metabolic activity in the tissues (hydrochloric, sulfuric, phosphoric and lactic acids) is removed from the body, as carbon dioxide, through the lungs. Likewise, since CO_2 is being constantly formed through oxidative processes in the body, any excess of alkali is rapidly transformed into bicarbonate.

Because of these remarkable neutralizing properties, the carbonic acid-bicarbonate system constitutes one of the most important buffer mechanisms of the body, operating particularly in the blood but to a lesser extent also in the tissue cells, the phosphate system, however, playing a more important rôle in the latter situation. Because of the fact that the blood bicarbonate represents a supply of base which is readily available for the neutralization of acids, to it has been applied the term "alkali reserve" of the blood, which, for clinical purposes, may be assumed to be representative of the alkali reserve of the body.

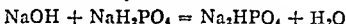
THE PHOSPHATE SYSTEM

The phosphate system consists of a mixture of monosodium phosphate and disodium phosphate. The hydrogen ion concentration of such a mixture, as in the case of the bicarbonate system, is dependent upon the relative proportion of each of these two substances. In other words $\text{cH} = \frac{\text{NaH}_2\text{PO}_4}{\text{Na}_2\text{HPO}_4}$, increasing and decreasing accordingly as this ratio is increased or decreased. Since monosodium phosphate is but weakly acid in reaction and disodium phosphate but weakly alkaline, variations in the ratio between these two substances cause but little alteration in the hydrogen ion concentration of the mixture. The buffer action of a phosphate system is manifested in essentially the same manner as is that of a bicarbonate system. The addition of a strongly acid substance results in the following reaction:



In this way the addition of a strong acid results in the formation of monosodium phosphate (NaH_2PO_4) which is but weakly acid

in reaction and gives rise to relatively few hydrogen ions with little consequent change in the hydrogen ion concentration of the solution. In a similar manner the addition of a strong alkali results in the following reaction:

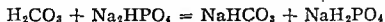


The strongly alkaline substance is thereby converted into disodium phosphate which is weakly alkaline, giving rise to relatively few OH ions with but little consequent change in the hydrogen-ion concentration of the mixture. The phosphate system operates particularly in the tissues and to a relatively small extent in the blood.

PLASMA PROTEINS, HEMOGLOBIN AND CHLORIDE

It is now recognized that the blood proteins, particularly hemoglobin, albumin and globulin, play an important part in regulating the hydrogen ion concentration of the blood. The plasma proteins are of much less significance than hemoglobin in this connection. Being amphoteric in nature, they act as weak acids in the blood plasma, the reaction of which is on the alkaline side of the isoelectric points of these substances which therefore combine with a small amount of base. The work of Van Slyke and his co-workers indicates that about $\frac{1}{10}$ of the buffer activity of the blood is due to plasma proteins. This constitutes so small a proportion of the total alkali-binding power of the blood that even marked diminution in the concentration of plasma proteins has practically no effect upon the efficiency of the buffer mechanism.

The mechanisms described above are effective in diminishing the disturbing effect of acids stronger than carbonic acid such as hydrochloric, sulfuric, phosphoric and lactic acids. However, H_2CO_3 , which is the acid formed in largest quantity in the body, cannot be efficiently dealt with by the bicarbonate system. The phosphate system may to a certain extent act as a buffer for carbonic acid according to the following equation:



However, since phosphates are present in such small quantity in the blood plasma and tissue fluids, they play a relatively unimportant part in this connection. By far the greater part of the base available for the neutralization of the large quantities of carbonic acid entering the blood from the tissues is supplied by hemoglobin and the blood chloride.

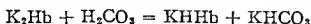
Hemoglobin, like other blood proteins, acts as a weak acid, combining with base which, in the interior of the red corpuscles,

is chiefly potassium, forming salts of reduced hemoglobin and oxyhemoglobin (KHB and KHBO_2). Oxyhemoglobin is much more strongly acid than reduced hemoglobin. The wall of the red corpuscle is normally impermeable to hemoglobin, sodium (Na) and potassium (K) but is freely permeable to chloride (Cl) and carbonic acid (H_2CO_3). Consequently the various ions of the blood will be distributed between the plasma and the interior of the red cells in accordance with the laws governing the forces operating to produce a Donnan equilibrium (see p. 230). The following substances will be present in the interior of the red corpuscles: H_2CO_3 , K_2HB , KCl and KHCO_3 ; the following will be present in the blood plasma: H_2CO_3 , NaCl and NaHCO_3 .

Plasma	Cells
CO_2 \swarrow $\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3$ NaCl NaHCO_3	CO_2 \swarrow $\text{CO}_2 + \text{H}_2\text{O} = \text{H}_2\text{CO}_3$ $\text{K}_2(\text{Hb})$ KCl KHCO_3

Fig. 9.—Entrance of CO_2 into plasma and cells.

According to Donnan's law the ratio, $\frac{\text{HCO}_3(\text{corpuscles})}{\text{HCO}_3(\text{plasma})}$, should equal the ratio, $\frac{\text{Cl}(\text{corpuscles})}{\text{Cl}(\text{plasma})}$, when the system is at equilibrium. When large quantities of carbonic acid enter the blood stream, as in the tissues, the greater part passes into the red corpuscles where, being a relatively stronger acid than hemoglobin, it reacts with the potassium salt of reduced hemoglobin according to the following equation:



The condition now existing in the corpuscles and plasma may be illustrated in the following diagram (Fig. 10).

Plasma	Cells
NaCl NaHCO_3 H_2CO_3	$\text{K}_2(\text{Hb}) + \text{H}_2\text{CO}_3 = \text{KH}(\text{Hb}) + \text{KHCO}_3$ KCl H_2CO_3 KHCO_3

Fig. 10.—Reaction of excess H_2CO_3 with $\text{K}_2(\text{Hb})$.

There is now in the interior of the red cell an excess of HCO_3 ions, which temporarily disturbs the equilibrium between bicarbonate and chloride ions in the red cells and plasma. Accordingly, some of the excess HCO_3 passes out of the red cells into the plasma, an equivalent concentration of Cl passing from the plasma into the red cells until the concentration equilibrium of these two ions is restored.

Conversely, as carbonic acid is lost from the blood in its passage through the lungs, diffusion of HCO_3 and Cl takes place in the opposite directions, the former passing from the plasma into the red corpuscles and the latter from the corpuscles into the plasma. This diffusion of Cl between the blood plasma and the interior of the red cell is termed the "chloride shift." Van Slyke has estimated that from 84–90 per cent of the base which is required to deal with H_2CO_3 is supplied directly or indirectly

Plasma	Cells
$\text{Na}(\text{Cl})$ NaHCO_3 H_2CO_3	$\text{K}(\text{HCO}_3)$ $\text{KH}(\text{Hb})$ KCl H_2CO_3

Fig. 11.—"Chloride shift." Exchange of Cl and HCO_3 between plasma and cells.
(After Peters and Van Slyke.)

by hemoglobin, the indirect supply being derived from the NaCl of the blood plasma, the buffering action of the hemoglobin, however, being essential for this reaction.

The reactions which take place in the blood in the lungs and tissues may be depicted in the following manner. In the lungs, venous blood is exposed to a relatively low CO_2 and a relatively high O_2 tension. Consequently, HCO_3 leaves and O_2 enters the red cells; as a result of the loss of HCO_3 from the corpuscles, Cl diffuses from the red cells into the plasma, restoring the concentration equilibrium. In consequence of these changes there is an increase in plasma chloride, a decrease in plasma bicarbonate and a decrease in corpuscular chloride. In the tissues, on the other hand, where the arterial blood is exposed to a relatively high CO_2 tension and a relatively low O_2 tension, oxygen passes out of and carbonic acid into the red cells. As a result of the increased concentration of HCO_3 in the interior of the red corpuscles, Cl diffuses from the plasma into the cells, the end-result being a decrease in plasma chloride, an increase in plasma bicarbonate

and an increase in corpuscular chloride. These changes are facilitated by the alterations in the acidity of hemoglobin which occur as a result of its oxygenation and reduction, oxyhemoglobin being about seventy times as strongly acid in reaction as reduced hemoglobin.

ALKALI RESERVE

The term "alkali reserve" is applied to the supply of base available for participation in all reactions whereby the acidifying effect of relatively strong acids entering from without or formed during metabolic processes is diminished or buffered, the hydrogen ion concentration of the tissue fluid being maintained within normal limits. This reserve supply consists not only of base present in the blood in combination with bicarbonate, phosphate, plasma protein, hemoglobin and chloride but also that present in perhaps similar combinations in the tissue fluid and the tissue cells themselves. However, clinically, the term "alkali reserve" is commonly applied to that portion of the base present in the form of bicarbonate in the blood plasma. This conception of the alkali reserve is satisfactory for all practical purposes since the concentration of blood bicarbonate is fairly representative of the state of the total alkali reserve of the body.

The mechanisms described above prevent the occurrence of sudden variations in the hydrogen ion concentration of the blood and tissues which would in their absence occur as a result of the entrance or elaboration of acid substances. However, if no other provision were made, the available alkali of the body would soon be used up or rendered unavailable by reason of its combination with these acids. This possibility is obviated by the action of certain excretory processes by means of which the excess acid is removed from the body through various channels.

EXCRETORY PROCESSES

The excess acid which must be removed from the body is eliminated chiefly through the lungs, gastro-intestinal tract and kidneys. Any excess of alkali is eliminated largely by the kidneys.

Lungs. The lungs are the chief channel of elimination of volatile acids, the most important of which is carbonic acid, the acid formed in largest quantity in the body. The activity of the respiratory center is governed by the hydrogen ion concentration within the cells constituting that center, which is dependent upon the hydrogen ion concentration of the blood and tissue fluid bathing those cells. The respiratory center is remarkably sensitive to very slight variations of the CO_2 tension and the pH of the blood. Haldane states that a rise of 0.2 per cent in the CO_2

pressure or a decrease of 0.012 in the pH of arterial blood results in an increase of about 100 per cent in the resting alveolar ventilation. This increased ventilation results in the washing out of the excess CO_2 and the restoration of the normal blood reaction. The extreme delicacy of this regulatory mechanism is indicated by the fact that this increase in acidity, although sufficient to double the resting ventilation, is too slight to be accurately detected by chemical or physiochemical methods. Conversely, an increase in the alkalinity of the blood, with very slight increase in the pH , diminishes the elimination of CO_2 by the lungs by diminishing the activity of the respiratory center with consequent diminution in the minute ventilation effected by a diminution in the rate and depth of respiration. This mechanism constitutes a most efficient means of maintaining the hydrogen ion concentration within normal limits.

Gastro-Intestinal Tract. The function of the gastro-intestinal tract in regulating the acid-base balance of the body is much less important than that of the lungs or kidneys. During the period of active gastric digestion large quantities of free hydrochloric acid are secreted into the stomach. There is some evidence to suggest that this is due not so much to an increased passage of chloride ion from the blood into the stomach as to diminished excretion of base, since the total chloride concentration of the gastric juice increases to a relatively slight degree during this period. These changes result in a relative increase in the quantity of base in the blood present in the form of bicarbonate, in consequence of which fact the organism attempts to restore the normal equilibrium by the excretion of urine of increased alkalinity. This phenomenon is designated the alkaline tide, which occurs during periods of active gastric digestion with the secretion of free hydrochloric acid into the stomach. With the completion of this period and a return of gastric acidity to the resting level, the free hydrochloric acid, having passed into the small intestine and having been neutralized by the bases there present, is reabsorbed into the blood stream and the bicarbonate and chloride concentrations of the plasma are restored to their normal levels. The urinary alkaline tide then disappears, the reaction of the urine becoming more acid.

Although the part played by the gastro-intestinal tract in the maintenance of the normal acid-base balance under physiologic conditions is relatively unimportant, in certain pathologic conditions affecting the alimentary canal marked alterations in the hydrogen ion concentration of the blood may occur. In this connection, the volume and electrolyte composition of the various digestive fluids are of importance. It has been estimated

that there are secreted in twenty-four hours approximately 1500 cc. of saliva, 2500 cc. of gastric juice, 500 cc. of bile, 700 cc. of pancreatic juice and 3000 cc. of intestinal secretions, a total of 8200 cc. of fluid. In view of the fact that the total plasma volume of an average adult is about 3500 cc., it is obvious that the continued loss of various digestive fluids can readily lead to a state of marked dehydration. Such loss of fluid inevitably involves a loss of electrolytes, and the resulting disturbance of acid-base balance depends largely upon the electrolyte composition of the lost secretions. This is presented in Fig. 5 (p. 227).

Kidneys. The kidneys play a most important part in the maintenance of the normal acid-base balance. Many fixed acids formed during metabolic processes are eliminated in the urine, chiefly in the form of salts of sodium, potassium, calcium, magnesium and ammonia (chlorides, phosphates, carbonates and sulfates). In addition, in the kidneys, the disodium phosphate of the blood plasma (Na_2HPO_4) is transformed into the acid phosphate (NaH_2PO_4) which contributes largely to the normal acidity of the urine. This phenomenon aids materially in conserving the available base of the blood and in counteracting any tendency toward an increase in the hydrogen ion concentration of the plasma. The variation in urinary acidity which occurs during periods of active gastric digestion (alkaline tide) has been discussed above. Excessive quantities of alkali present in the body are eliminated in the urine chiefly in the form of bicarbonate, there being a simultaneous diminution in the proportion of phosphate present in the form of the acid salt (BH_2PO_4). Likewise, the administration of mineral acids or acid-forming substances such as ammonium chloride results not only in the elimination of increased quantities of the ingested anion (chloride) but also in an increase in the amount and proportion of acid phosphate in the urine. Thus, under normal circumstances the kidneys constitute an extremely delicate mechanism for the elimination of excessive quantities of fixed acids and bases from the body, operating in a manner comparable in efficiency to the action of the respiratory mechanism in eliminating excess quantities of carbonic acid.

Formation of ammonia in the kidneys is another extremely important factor in the preservation of the normal acid-base balance. Excessive amounts of hydrochloric, sulfuric, lactic and other acids, either ingested or formed during metabolic processes, must be eliminated in the urine in combination with basic radicles in a partially or completely neutralized form. In the absence of any base-conserving process this elimination would result in the removal from the body of relatively large quantities

of alkali, such as sodium, potassium, calcium and magnesium, with consequent depletion of the alkali reserve. Ammonia, formed in the kidney from amino acids, combines with these acid radicals and thus conserves the available base supply of the body. A normal adult eliminates from 0.5 to 1.0 Gm. of ammonia nitrogen in the urine daily, constituting from 2-5 per cent of the total urinary nitrogen. The ammonia-forming mechanism is stimulated by the necessity for the elimination of increased quantities of acids other than carbonic and phosphoric and therefore the urinary ammonia is increased following the ingestion or formation in the body of increased amounts of acid substances. Conversely, the necessity for eliminating increased quantities of basic substances is associated with a depression of ammonia formation and consequent diminution in urinary ammonia. The ammonia-forming mechanism constitutes a most important factor in the conservation of the base supply of the body. The important role of the kidney in defending the chemical pattern of the blood plasma and other extracellular fluids of the body is indicated in Fig. 13, p. 343.

PATHOLOGIC CONSIDERATIONS

It is obvious from a consideration of the preceding discussion that pathologic changes in the acid-base balance may result from disturbances in one or more of several factors involved in the maintenance of the normal equilibrium. For the sake of convenience, however, most pathologic alterations in the acid-base balance may be considered from the standpoint of changes in the ratio of the concentration of carbonic acid to that of bicarbonate, since, in the majority of conditions observed clinically, the state of the bicarbonate system reflects fairly accurately the condition of the acid-base balance of the entire body. In other words, in dealing with the hydrogen ion concentration of the blood, it may be considered that

$$cH = K \frac{H_2CO_3}{BHCO_3},$$

K being a constant and B constituting the so-called "alkali reserve," consisting chiefly of sodium and potassium. Obviously, these changes in the hydrogen ion concentration of the blood may result from changes in the concentration of either carbonic acid or bicarbonate and changes of equal magnitude in the same direction may occur in these two fractions with no associated alteration in the hydrogen ion concentration. Disturbances of the normal acid-base equilibrium are termed

"acidosis" and "alkalosis" accordingly as the hydrogen ion concentration tends to increase or decrease.

ACIDOSIS

The term "acidosis" is applied to the condition resulting from the formation or absorption of acids at a rate exceeding that of their neutralization or elimination. It may also, although much less frequently, be due to the loss of excessive quantities of base from the body. On the basis of the equation,

$$cH = K \frac{H_2CO_3}{BHCO_3},$$

it is evident that an increase in the hydrogen ion concentration of the blood (acidosis) may be caused by either an increase in the concentration of H_2CO_3 or a decrease in the concentration of $BHCO_3$. If these changes are of such magnitude that the hydrogen ion concentration rises above the upper limit of normal (below 7.3) the condition is one of uncompensated acidosis. However, as has been indicated above, because of the existence of a remarkably efficient compensatory mechanism, if the concentration of carbonic acid rises, the concentration of blood bicarbonate also tends to increase in order to maintain the normal equilibrium; likewise, as the concentration of blood bicarbonate diminishes, increased quantities of carbon dioxide are removed through the lungs with a consequent compensatory decrease in the concentration of carbonic acid in the blood. Because of these compensating processes, primary alterations in either of these two factors are, for a time at least, balanced to a certain degree by secondary changes in the other factor, as a result of which there may be little or no perceptible alteration in the hydrogen ion concentration. If under such circumstances the hydrogen ion concentration of the blood is maintained below the upper limit of normal (pH above 7.3) the organism is in a state of compensated acidosis. The clinical conditions in which a state of acidosis is commonly observed will be considered under two headings: (1) those associated with a primary increase in the concentration of H_2CO_3 in the blood; (2) those associated with a primary decrease in blood bicarbonate (alkali reserve).

Primary H_2CO_3 Excess. Increase in the carbonic acid content (CO_2 tension) of the blood may occur in one of two general ways:

(1) Rebreathing, or breathing air containing abnormally high percentages of CO_2 .

(2) Conditions in which the elimination of CO_2 through the lungs is retarded. In this group may be placed conditions causing

of alkali, such as sodium, potassium, calcium and ... with consequent depletion of the alkali reserve. Ammonia formed in the kidney from amino acids, combines with the acid radicals and thus conserves the available base supply of the body. A normal adult eliminates from 0.5 to 1.0 Gm. of ammonia nitrogen in the urine daily, constituting from 2-5 per cent of the total urinary nitrogen. The ammonia-forming mechanism is stimulated by the necessity for the elimination of increased quantities of acids other than carbonic and ... and therefore the urinary ammonia is increased following the ingestion or formation in the body of increased amounts of such substances. Conversely, the necessity for eliminating increased quantities of basic substances is associated with a depression of ammonia formation and consequent diminution in urinary ammonia. The ammonia-forming mechanism constitutes an important factor in the conservation of the base supply of the body. The important role of the kidney in defending the normal chemical pattern of the blood plasma and other extracellular fluids of the body is indicated in Fig. 13, p. 343.

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Primary Alkali Deficit. Primary alkali deficit is the most common cause of acidosis occurring in clinical conditions. In this group may be placed the following conditions:

DIABETES MELLITUS (see p. 338). Acidosis due to alkali deficit is a constant feature of advanced diabetes mellitus. It is due in part to the existing ketosis and in part to the excessive loss of water with the associated elimination of excessively large quantities of base. As demonstrated by Atchley, the polyuria which attends the development of glycosuria is accompanied by a pronounced increase in the excretion of electrolytes, particularly sodium, potassium and chloride. This initial electrolyte loss is supplemented by a secondary increase in the loss of water and electrolytes simultaneously with the development of ketosis. These changes eventually lead to dehydration and depletion of body base. Peters regards the following concurrent phenomena as constituting the mechanism underlying the production of changes in the electrolyte pattern of the blood in diabetes: (1) Displacement of CO_2 from bicarbonate by ketone acids. (2) Although a portion of the ketone acids can be eliminated as free acid and a portion is neutralized by ammonia, a certain fraction is neutralized by fixed base. The excretion of the latter fraction, in contradistinction to the former, withdraws base from the body which can be replaced only from extraneous sources. (3) Reduction of base signifies reduction of the total electrolyte content of the body fluids and, consequently, dehydration, resulting from the simultaneous loss of body fluids. (4) Chloride depletion is apparently related more directly to glycosuria and polyuria than to acidosis.

The mechanism of production of ketosis in diabetes mellitus is discussed in detail elsewhere (p. 336). Regardless of whether or not this results from incomplete oxidation of fatty acids in the tissues or excessive production of ketones in the liver because of deficient glycogen content of the hepatic cells, this disturbance of fat metabolism in diabetes is secondary to the fundamental disturbance in carbohydrate metabolism. The ketone substances, including aceto-acetic acid, betahydroxybutyric acid and acetone, accumulate in the blood and tissues, the first two, being acid in reaction, combining with a portion of the fixed base supply and thus diminishing the alkali reserve of the body.

RENAL FAILURE. Acidosis is commonly observed in the terminal stages of nephritis and destructive renal lesions such as polycystic kidney, hydronephrosis, pyonephrosis, pyelonephritis and renal tuberculosis. It is due in part to the fact that the impairment of renal functional efficiency eventually causes retention of those acid radicles which are normally

mechanical asphyxia, morphine narcosis with its associated diminished minute ventilation, pneumonia, particularly bronchopneumonia, pulmonary emphysema, in which the diffusion of CO_2 is impaired, and cardiac decompensation, in which pulmonary congestion and slowed circulation combine to cause increased CO_2 tension in the blood.

Compensatory Mechanisms. As the carbonic acid content of the blood increases, certain compensatory mechanisms come into play by means of which the organism attempts to maintain the hydrogen ion concentration of the blood within normal limits.

(1) INCREASED VENTILATION. The increased stimulation to the respiratory center caused by the increased CO_2 tension of the blood results in increased depth and rate of respiration with consequent increased ventilation. As stated above, the sensitivity of the respiratory center to a relatively slight increase in hydrogen ion concentration or CO_2 tension of the blood renders this mechanism extremely efficient in counteracting the effects of primary CO_2 excess.

(2) INCREASE IN ALKALI RESERVE. In conditions associated with primary CO_2 excess the blood bicarbonate has been found to be increased. This simultaneous change in both elements of the bicarbonate system tends to prevent the increase in hydrogen ion concentration of the blood which would otherwise occur. The increase in blood bicarbonate is due in part to the effect of the increased carbonic acid content in causing displacement of the chloride ion from the blood chloride (chloride shift), the excess chloride being in all probability eliminated in the urine as ammonium chloride.

(3) INCREASED AMMONIA FORMATION. An increase in the rate of ammonia formation and excretion by the kidney tends to diminish the loss of base from the body and to conserve the alkali reserve.

(4) INCREASED URINARY ACIDITY. Increased quantities of acid radicles are eliminated in the urine, the proportion of phosphate present as the acid salt (NaH_2PO_4) being greater than under normal conditions.

Because of the efficiency of these compensatory mechanisms, primary CO_2 excess rarely results in a state of uncompensated acidosis as evidenced by the hydrogen ion concentration of the blood. From this standpoint, therefore, conditions in this group are of relatively minor importance clinically, symptoms of acidosis, apart from dyspnea, being encountered only infrequently and disturbance of the acid-base balance being rarely of sufficient magnitude to be of serious import.

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eliminated in the urine, among the most important being phosphoric and sulfuric acids. Nephritic acidosis is also contributed to by the accumulation of certain organic acids of undetermined nature. As these acids accumulate in the blood and tissues they fix and render unavailable a portion of the base supply of the body, causing a diminution in and eventual depletion of the alkali reserve. Two other factors play an important part in contributing to the acidosis of advanced nephritis. The body appears to have lost its ability to conserve base, which is lost in the urine and at times through the gastro-intestinal tract (vomiting). Furthermore, the ability of the kidney to form ammonia is diminished so that this mechanism, which in the presence of normal renal function is capable of handling increased quantities of acid substances and thus aids in the conservation of the alkali reserve, fails to counteract the acidotic tendency associated with renal failure.

STARVATION. Starvation and carbohydrate restriction are associated with a state of acidosis due to ketosis. The underlying mechanism, namely, incomplete fatty acid combustion, is identical with that occurring in diabetes mellitus, being dependent in the one case upon perfect utilization of insufficient quantities of glucose and in the other upon imperfect glucose utilization.

ANESTHESIA. The alkali reserve is diminished during anesthesia produced by ether and chloroform and, to a lesser degree, ethylene and nitrous oxide. It is believed by some that this may be due to the accumulation of excessive quantities of lactic acid as a result of anoxemia and imperfect glucose combustion. Ketosis and ketonuria frequently occur in the later stages of anesthesia and for some time after, but in all probability do not contribute in an important measure to the development of acidosis during the earlier period of anesthesia.

DEHYDRATION (p. 255). The loss of excessive quantities of water from the body is invariably associated with the coincident or subsequent loss of a proportional amount of electrolytes, for the concentration of the latter in the body fluids must be maintained within rather narrow limits. Accordingly dehydration, if of extreme grade, is commonly associated with a state of acidosis due to the excessive loss of base from the body. This is particularly true of conditions in which the lost fluid contains relatively large quantities of base and is alkaline in reaction. For example, in diarrhea and in intestinal or pancreatic fistulae large quantities of fluid, containing large amounts of base, are removed from the body with consequent dehydration and acidosis due to primary alkali deficit (p. 236). In dehydration due to

excessive vomiting the matter is complicated by the fact that, the gastric contents being normally acid in reaction, large quantities of acid (HCl) are lost from the body, thus balancing the alkali deficit which might otherwise occur. As a matter of fact, alkalosis (alkali excess) is the most common sequel of excessive vomiting under ordinary conditions except in cases of achlorhydria or achylia. The inanition and carbohydrate restriction which are necessarily of common occurrence in conditions of continued vomiting may contribute to the production of alkali deficit because of the associated starvation ketosis. In a certain proportion of normal individuals there may be little or no free hydrochloric acid in the gastric juice, and continued vomiting, producing dehydration in such individuals, will eventually result in acidosis. Furthermore, in the vomiting of advanced nephritis (uremia) the gastric juice usually contains little or no free hydrochloric acid due to the fact that the organism seems to have lost its ability to conserve base, and under such conditions the acidosis of dehydration may aggravate that already existing as a result of advanced renal insufficiency. Acidosis is frequently an important feature of the continued recurrent or cyclic vomiting of children. The loss of hydrochloric acid from the stomach does not appear to counteract effectively the acidotic tendency in this condition, the alkali deficit being apparently due to the formation of excessive quantities of organic acids, particularly the ketone acids and lactic acid.

INGESTION OF ACIDS. Acidosis due to alkali deficit may be produced by the administration of excessive quantities of such substances as HCl, H_3PO_4 , NH_4Cl and similar agents.

PREGNANCY AND TOXEMIAS OF PREGNANCY (see pp. 518, 521). The later stages of normal pregnancy are associated with a state of compensated acidosis due to slight alkali deficit, the cause of which has not been determined. In eclampsia, particularly during and shortly after convulsive seizures, the organism is in a state of uncompensated acidosis, pH values ranging from 7 to 7.2 having been observed in some instances. In the toxemias of early pregnancy, characterized by excessive and persistent vomiting, the acid-base balance is in a state similar to that existing in persistent vomiting due to other causes, acidosis being, however, much more frequently observed than alkalosis, as in the case of persistent vomiting in infants.

Compensatory Mechanisms. As the alkali reserve of the body diminishes certain compensatory mechanisms are set in operation in an attempt to maintain the hydrogen ion concentration within normal limits.

(1) **INCREASED PULMONARY VENTILATION.** The respiratory

center responds to an increase in hydrogen ion concentration due to primary alkali deficit just as it does to increased CO_2 tension, namely, by causing increased rate and depth of respiration. As a consequence of this increased pulmonary ventilation, CO_2 is washed out of the blood and the ratio of blood carbonic acid to blood bicarbonate tends to be restored to normal, the hydrogen ion concentration being consequently but little affected. During the early stages of alkali deficit, therefore, the organism is in a state of compensated acidosis, but as the condition progresses and the alkali deficit becomes more pronounced the compensatory mechanism fails and the condition becomes one of uncompensated acidosis with an increase in the hydrogen ion concentration of the blood.

(2) INCREASED AMMONIA FORMATION. As the alkali reserve diminishes the organism attempts to conserve the base supply by increased ammonia formation in the kidney. This ammonia combines with the excessive amounts of acid radicles which must be eliminated in the urine, thus preventing the loss from the body of a portion of its alkali reserve (sodium, potassium, calcium and magnesium). In nephritis, however, this mechanism fails to a certain degree, due to the fact that the ammonia-forming function of the kidney is impaired, perhaps proportionately to the impairment in its excretory function. Furthermore, as stated above, in the presence of renal failure the ability of the body to conserve base appears to be impaired, with the result that excessive quantities of base are eliminated in the urine.

(3) INCREASED ACID EXCRETION. In the majority of cases of acidosis due to primary alkali deficit increased quantities of acid, chiefly acid phosphate (NaH_2PO_4), are eliminated in the urine. This does not occur in the acidosis of advanced nephritis which is in itself partly due to failure of elimination of acid phosphate by the kidneys. The increased elimination of electrolytes in the urine and the diminution in the concentration of alkali in the blood and tissue fluids are invariably associated with the elimination of increased quantities of water in an attempt to maintain the normal electrolyte concentration of the blood and tissue fluids. This diuresis results in dehydration which is one of the constant features of this type of acidosis.

ALKALOSIS

Alkalosis is a state in which either excessive amounts of acid are lost from the body without a comparable loss of base or alkali, or alkali is formed in or supplied to the body at a rate exceeding that of its neutralization or elimination. In terms of the bicarbonate system, alkalosis may result from either a

primary decrease in the carbonic acid of the blood or a primary increase in blood bicarbonate (alkali reserve). As in the case of acidosis, a primary change in one of these factors is almost invariably associated with or followed by a secondary change in the same direction in the other factor, the hydrogen ion concentration of the blood under these circumstances being but little affected, the condition being one of compensated alkalosis. As the metabolic error progresses, however, the compensatory secondary change becomes insufficient to maintain the normal balance and the hydrogen ion concentration of the blood diminishes below the lower limit of normal (pH above 7.5), constituting a state of uncompensated alkalosis.

Primary H_2CO_3 Deficit. Excessive quantities of CO_2 may be washed out of the blood by hyperventilation of the pulmonary alveoli. This condition may be induced voluntarily by excessively rapid and deep respiration. Clinically, it is observed in the following conditions:

HYSTERIA. Alkalosis due to primary H_2CO_3 deficit is occasionally observed as a result of the hyperventilation which occurs at times during hysterical attacks.

FEVER. Hyperventilation may occur as a result of the increased respiratory rate associated with an increase in body temperature. This is particularly prone to occur in inflammatory conditions involving the respiratory passages, especially pneumonia, in which condition the tendency toward H_2CO_3 deficit is balanced by a tendency toward retention of H_2CO_3 due to diminution in and functional impairment of the pulmonary ventilating surface.

HIGH EXTERNAL TEMPERATURES. Hyperventilation may be induced by exposure to high external temperatures such as hot baths. If prolonged, this exposure may result in alkalosis due to primary H_2CO_3 deficit.

ANOXIC ANOXEMIA. Hyperpnea occurring in untrained individuals ascending to high altitudes where the atmospheric oxygen tension is low (anoxic anoxemia) commonly results in primary H_2CO_3 deficit and alkalosis.

ENCEPHALITIS. Alkalosis due to hyperventilation has been observed in some cases of encephalitis manifesting hyperpnea over prolonged periods of time.

Compensatory Mechanisms. (1) **EXCRETION OF ALKALI.** As the hydrogen ion concentration of the blood diminishes an increased quantity of alkali, in the form of bicarbonate, is eliminated in the urine.

(2) **DECREASED ACID ELIMINATION.** There is a diminution in the proportion of phosphate present in the urine in the form of

the acid salt (NaH_2PO_4) with a consequent increase in the excretion of alkaline phosphate (Na_2HPO_4).

(3) **DECREASED URINARY AMMONIA.** The elimination of base in the urine is further enhanced by a diminution in the formation of ammonia by the kidney.

(4) **RETENTION OF ACID METABOLIC PRODUCTS.** As the condition of alkalosis progresses, ketone bodies (diacetic and betahydroxybutyric acid and acetone) may accumulate in the blood due, presumably, to a disturbance of fatty acid oxidation. Ketonuria may occur under such circumstances. This ketosis, however, has but little effect upon the acid-base equilibrium.

Primary Alkali Excess. Primary alkali excess or increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. It occurs in the following conditions:

EXCESSIVE LOSS OF HCL FROM THE STOMACH. The loss of excessive quantities of hydrochloric acid from the stomach is encountered most frequently in individuals with pyloric or high intestinal obstruction and following protracted gastric lavage without proper provision for acid replacement. It is also at times observed in infants with pylorospasm and in patients with generalized peritonitis. As a result of the loss of the Cl ion from the blood there is present in the body an excess of base, chiefly sodium and potassium, which is retained in the form of bicarbonate. In this way a neutral salt (NaCl) is replaced by an alkaline salt (NaHCO_3). Alkalosis due to primary alkali excess is one of the most constant metabolic features of pyloric or upper intestinal obstruction, this disturbance of the acid-base balance, together with hypochloremia and nitrogen retention, constituting important diagnostic features of those conditions (see pp. 101, 235, 254).

ALKALI ADMINISTRATION. Alkalosis may follow the administration of excessive amounts of alkaline substances, particularly sodium bicarbonate, which is frequently given in large doses in the treatment of peptic ulcer and as a routine postoperative procedure. The administration of sodium bicarbonate in the treatment of acidosis in chronic nephritis is particularly liable to result in alkalosis due to the difficulty of elimination of the excess alkali by the diseased kidneys.

ROENTGEN RAY AND ULTRAVIOLET IRRADIATION AND RADIUM THERAPY. A decrease in the hydrogen ion concentration of the blood plasma (increased pH) has been observed following deep x-ray therapy, radium therapy and prolonged exposure to ultraviolet rays. This decrease in hydrogen ion concentration is associated with a diminution in the concentration of serum phosphate. In some instances the plasma chloride content is

subnormal, a factor which may contribute to the production of alkalosis by releasing base for combination with bicarbonate.

Compensatory Mechanisms. (1) INCREASED ALKALI EXCRETION. In most cases of alkalosis due to alkali excess the urine contains an increased quantity of base, principally in the form of sodium bicarbonate. In some instances, in spite of the presence of uncompensated alkalosis, the urine remains acid, due perhaps, to functional impairment of renal excretory activity.

(2) DECREASED ACID EXCRETION. The urine contains a diminished quantity of phosphate present in the form of the acid salt (NaH_2PO_4) and a relatively large quantity in the form of the alkaline salt (Na_2HPO_4).

(3) DECREASED AMMONIA FORMATION.

(4) RETENTION OF ACID METABOLIC PRODUCTS (KETOSIS).

(5) DECREASED PULMONARY VENTILATION. As the hydrogen ion concentration of the blood diminishes the activity of the respiratory center is depressed with consequent diminution in the rate and depth of respiration. As a result of this phenomenon there is a decrease in the rate of elimination of CO_2 by the lungs with a consequent tendency toward retention of CO_2 (H_2CO_3) in the blood.

METHODS OF STUDYING ACID-BASE BALANCE

The difficulty of investigating accurately the condition of a system which may be disturbed in one or more of so many ways must be apparent. Perhaps the most satisfactory method of approach, from a clinical standpoint, is to consider all disturbances of the acid-base balance in terms of the bicarbonate system, as has been done above, and to consider the hydrogen ion concentration of the blood as dependent upon the ratio between the concentrations of carbonic acid and bicarbonate in the blood. In other words,

$$\text{cH} = K \frac{\text{H}_2\text{CO}_3}{\text{BHCO}_3}$$

It must be remembered that in most cases a primary change in the concentration of either H_2CO_3 or BHCO_3 is associated with or followed by a secondary compensatory change in the same direction in the other factor, in an attempt to maintain the hydrogen ion concentration within normal limits. For example, if the BHCO_3 concentration (alkali reserve) is diminished, as in diabetes mellitus and nephritis, the development of uncompensated acidosis with an increase in the hydrogen ion concentra-

tion of the blood is for a time prevented by a compensatory decrease in the H_2CO_3 concentration of the blood (compensated acidosis). The subnormal carbonic acid content of the blood is in this instance a manifestation of acidosis. On the other hand, a primary decrease in the H_2CO_3 concentration is observed in conditions associated with hyperventilation, such as occurs at high altitudes, in fevers, encephalitis, hysteria, etc., resulting in a tendency toward a decrease in the hydrogen ion concentration which is for a time balanced by a compensatory secondary diminution in blood bicarbonate (alkali reserve). In this case the decreased bicarbonate and carbonic acid concentrations of the blood are indicative of a state of alkalosis (compensated). It is evident that a clear-cut distinction must be made between primary and secondary changes in these factors, primary increase in carbonic acid and primary decrease in bicarbonate being indicative of a state of acidosis whereas secondary increase in carbonic acid and decrease in bicarbonate are indicative of a state of alkalosis. On the other hand, primary decrease in carbonic acid and primary increase in bicarbonate are indicative of a state of alkalosis whereas a secondary decrease in carbonic acid or increase in bicarbonate is indicative of a state of acidosis.

In considering the problem from this standpoint, three variable factors must be considered: (1) The hydrogen ion concentration of the blood, (2) the H_2CO_3 concentration (CO_2 tension) and (3) the BHCO_3 concentration (alkali reserve). Obviously, a distinct disturbance of the acid-base balance may exist, as evidenced by a primary change in the concentration of either H_2CO_3 or BHCO_3 , which, by virtue of a compensatory change in the other factor, is associated with no significant alteration in the hydrogen ion concentration. In other words, the primary disturbance is compensated. Under such conditions the true state of the acid-base balance can be determined accurately only by the determination of at least two of the three components of the equation cited above (hydrogen ion concentration, blood carbonic acid and blood bicarbonate). Fortunately, however, in the great majority of clinical conditions associated with significant disturbances of the acid-base balance the fault lies primarily in a decrease or an increase in blood bicarbonate (alkali reserve), so that for most practical purposes investigation of this factor furnishes satisfactory although not exact information as to the state of the acid-base balance. It must be realized, however, that serious error may result from a failure to differentiate clearly between primary and secondary changes and that in doubtful cases too much reliance should not be placed upon the determination of any single factor.

ALKALI RESERVE OF THE BLOOD

For clinical purposes it may be assumed that changes in the alkali reserve of the body are reflected in the bicarbonate concentration (alkali reserve) of the blood.

Carbon Dioxide Capacity of the Plasma. The carbon dioxide capacity or CO_2 combining power of the blood plasma is expressed as the number of cubic centimeters of CO_2 which can be bound as bicarbonate by 100 cc. of blood plasma at 0°C . and 760 mm. Hg. pressure. Inasmuch as the ability of the plasma to combine with CO_2 to form bicarbonate depends, in the final analysis, upon the quantity of available alkali present (alkali reserve), the determination of the CO_2 combining power or capacity of the blood plasma is a direct measure of the alkali reserve. The normal values for adults range from 55 to 80 cc. of CO_2 bound as bicarbonate by 100 cc. of blood plasma (55-80 volumes per cent). The normal values for infants are about 10 volumes per cent lower than those for adults. Decrease or increase in the CO_2 combining power is indicative of a corresponding change in the alkali reserve.

Decrease in the CO_2 combining power, if but slight, is usually well compensated so that values ranging from 55 to 40 volumes per cent, indicative of a mild degree of acidosis, are generally associated with no significant alteration in the hydrogen ion concentration of the blood and consequently are not associated with clinical manifestations of acidosis. As the alkali deficit becomes more pronounced and the value for the CO_2 combining power drops to within the range of 40 to 30 volumes per cent, indicative of a state of moderate to severe acidosis, the compensatory mechanisms usually begin to fail, the acidosis becomes uncompensated, the hydrogen ion concentration is slightly increased and symptoms of acidosis are usually apparent. Values below 30 volumes per cent indicate a state of severe acidosis, practically always uncompensated, and therefore associated with a distinct increase in the hydrogen ion concentration. Values as low as 10 volumes per cent have been observed in individuals with eclampsia, severe diabetes and advanced nephritis (uremia), figures below 15 volumes per cent being usually indicative of a rapidly fatal termination.

Increase in the CO_2 combining power dependent upon primary alkali excess indicates a state of alkalosis. With values ranging from 80 to 90 volumes per cent the condition is usually well compensated, the hydrogen ion concentration of the blood being maintained within normal limits. However, since the great majority of the substances formed during metabolic activity in the tissues are acid in reaction, the compensatory mechanisms

of the body which operate to maintain the normal acid-base balance are apparently much more efficient in dealing with a tendency toward acidosis than in preventing a change in the direction of alkalosis. Consequently, primary increase in the alkali reserve results in a state of uncompensated alkalosis more readily than uncompensated acidosis is effected by a change in the opposite direction. Therefore, as the CO_2 combining power rises above 90 volumes per cent, indicating a state of moderate to severe alkalosis, the condition rapidly becomes uncompensated and the hydrogen ion concentration of the blood diminishes. In advanced alkalosis, such as is observed in some cases of pyloric and upper intestinal obstruction and in individuals receiving excessively large doses of alkali, the CO_2 combining power may reach 125 volumes per cent, although values above 110 are unusual.

Variation in the CO_2 combining power due to secondary compensatory changes in blood bicarbonate must be carefully distinguished from primary changes in this factor. A slight increase in the CO_2 combining power is commonly observed in acidosis associated with conditions in which there is a primary increase in blood carbonic acid, such as asphyxia, morphine narcosis, emphysema, etc. Similarly, a slight decrease in the CO_2 combining power may be observed in alkalosis dependent upon a primary decrease in blood carbonic acid, such as in hyperventilation of high altitudes, hysteria, encephalitis, etc. However, these conditions of primary H_2CO_3 excess and deficit are not commonly encountered clinically and are usually recognized without much difficulty. The great majority of clinical conditions associated with disturbance of the acid-base balance, such as advanced nephritis, diabetes mellitus, pyloric and acute intestinal obstruction, alkali overdosage, x-ray irradiation, etc., fall under the headings of primary alkali deficit or primary alkali excess. In these conditions the determination of the CO_2 combining power of the blood plasma is perhaps the most valuable single means of estimating the degree of acidosis or alkalosis in most instances. Although the values obtained by this method may be interpreted quantitatively in a manner satisfactory for all clinical purposes, they do not always indicate accurately the true quantitative variation in blood bicarbonate. Extremely high or extremely low values may apparently indicate a degree of alkalosis or acidosis of greater or less severity than is actually present. This does not detract, however, from the clinical value of this determination.

Plasma Bicarbonate. The bicarbonate concentration of the blood plasma may be determined directly by titration. Results

obtained by this method are, within a wide range of values, in close agreement with those obtained by the determination of the CO_2 combining power of the blood plasma. The titration method is more accurate for extremely low or extremely high values but for clinical purposes offers no advantage over the simpler and more available CO_2 capacity determination.

DETERMINATION OF CARBONIC ACID OF BLOOD

The H_2CO_3 content of the blood may be determined directly by analysis of the blood or indirectly by the determination of the CO_2 content of alveolar air which is in equilibrium with the CO_2 of arterial blood.

H_2CO_3 Content of Blood. The determination of the carbonic acid content of venous blood is of little value since it varies in different portions of the body in accordance with the metabolic activity in that region at the time the blood is drawn. Because of this fact and because arterial blood is not ordinarily employed for routine clinical purposes the direct method is seldom employed clinically for the determination of the carbonic acid content of the blood.

Alveolar CO_2 Tension. The determination of the H_2CO_3 content of the blood is of particular value in those conditions associated with primary H_2CO_3 excess or deficit. In certain of these conditions the determination of the CO_2 content of alveolar air may be employed to advantage. However, in pneumonia and emphysema, in which conditions primary H_2CO_3 excess in the blood, if present, is dependent upon imperfect diffusion through an altered respiratory membrane, the CO_2 content of the alveolar air cannot be assumed to be identical with that of arterial blood and hence indirect methods of determination of this factor should not be employed in these disorders. The chief clinical value of this method lies in the fact that the alveolar CO_2 content serves indirectly as an index of the concentration of blood bicarbonate (alkali reserve), since any increase or decrease in the latter factor, by causing depression or stimulation of the activity of the respiratory center with consequent hypoventilation or hyperventilation as the case may be, results in a compensatory increase or decrease in the concentration of carbonic acid in the blood and therefore in the CO_2 content of alveolar air.

The method of Marriott is perhaps most widely employed clinically. This procedure involves the collection and examination of rebreathed air, which differs from true alveolar air in that it has come into equilibrium with venous blood in the pulmonary capillaries whereas true alveolar air is in approximate equilibrium with arterial blood. The normal CO_2 values for men,

of the body which operate to maintain the normal acid-base balance are apparently much more efficient in dealing with a tendency toward acidosis than in preventing a change in the direction of alkalosis. Consequently, primary increase in the alkali reserve results in a state of uncompensated alkalosis more readily than uncompensated acidosis is effected by a change in the opposite direction. Therefore, as the CO_2 combining power rises above 90 volumes per cent, indicating a state of moderate to severe alkalosis, the condition rapidly becomes uncompensated and the hydrogen ion concentration of the blood diminishes. In advanced alkalosis, such as is observed in some cases of pyloric and upper intestinal obstruction and in individuals receiving excessively large doses of alkali, the CO_2 combining power may reach 125 volumes per cent, although values above 110 are unusual.

Variation in the CO_2 combining power due to secondary compensatory changes in blood bicarbonate must be carefully distinguished from primary changes in this factor. A slight increase in the CO_2 combining power is commonly observed in acidosis associated with conditions in which there is a primary increase in blood carbonic acid, such as asphyxia, morphine narcosis, emphysema, etc. Similarly, a slight decrease in the CO_2 combining power may be observed in alkalosis dependent upon a primary decrease in blood carbonic acid, such as in hyperventilation of high altitudes, hysteria, encephalitis, etc. However, these conditions of primary H_2CO_3 excess and deficit are not commonly encountered clinically and are usually recognized without much difficulty. The great majority of clinical conditions associated with disturbance of the acid-base balance, such as advanced nephritis, diabetes mellitus, pyloric and acute intestinal obstruction, alkali overdosage, x-ray irradiation, etc., fall under the headings of primary alkali deficit or primary alkali excess. In these conditions the determination of the CO_2 combining power of the blood plasma is perhaps the most valuable single means of estimating the degree of acidosis or alkalosis in most instances. Although the values obtained by this method may be interpreted quantitatively in a manner satisfactory for all clinical purposes, they do not always indicate accurately the true quantitative variation in blood bicarbonate. Extremely high or extremely low values may apparently indicate a degree of alkalosis or acidosis of greater or less severity than is actually present. This does not detract, however, from the clinical value of this determination.

Plasma Bicarbonate. The bicarbonate concentration of the blood plasma may be determined directly by titration. Results

during these periods evidence of a disturbance of the acid-base balance may be obtained by means of the estimation of the CO_2 combining power or alveolar CO_2 tension. The chief value of the determination of the hydrogen ion concentration of the blood in clinical conditions lies in the fact that when done in conjunction with one of the methods discussed above it indicates the degree of compensation or decompensation of the existing disturbance.

Values below pH 7.3 indicate a state of uncompensated acidosis, figures as low as 6.95 having been reported in advanced diabetes. Values below pH 7.0 are extremely rare, illustrating the fact that even in most severe acidotic states the blood practically always remains on the alkaline side of neutrality. Values above pH 7.5 indicate a state of uncompensated alkalosis, the highest values reported in clinical conditions being in the neighborhood of pH 7.6 although figures as high as pH 7.8 to pH 7.9 have been produced by experimental hyperventilation.

OTHER METHODS OF INVESTIGATION

Alkali Tolerance (Sellards' Test). The alkali tolerance test of Sellards depends in principle upon the fact that the administration of sodium bicarbonate to normal individuals in doses up to a maximum of 0.5 Gm. per kilogram of body weight causes the urine to become alkaline in reaction. In the majority of normal individuals the ingestion of 5-10 Gm. of sodium bicarbonate usually suffices to produce this effect. According to Van Slyke, in the presence of mild acidosis, 0.5-0.8 Gm. may be required; in moderate to severe acidosis 0.8-1.1 Gm. per kilogram of body weight are required; in severe acidosis more than 1.1 Gm. per kilo are necessary to produce an alkaline reaction in the urine. In an individual of 60 Kg. body weight, therefore, 0 to 30 Gm. may be considered to be normal, 30 to 50 Gm. may be assumed to indicate a state of mild acidosis, 50 to 65 Gm. moderate to severe acidosis and over 65 Gm. severe acidosis. The bicarbonate is administered in doses of 5 Gm., dissolved in a little water, every two or three hours until the urine, which is voided before each dose, becomes neutral or faintly alkaline to litmus, the urine being thoroughly boiled before testing.

This test is of more negative than positive value. If the urine becomes alkaline following the administration of less than 0.5 Gm. of sodium bicarbonate per kilogram of body weight it may be assumed that acidosis does not exist in that individual. However, higher values may be obtained in the absence of acidosis and the figures usually indicate a more severe grade of acidosis than actually exists. In other words, neither the presence

obtained by the Marriott method, range from 5.2 to 5.7 volumes CO_2 per cent, corresponding to a CO_2 tension of 40 to 45 mm. Hg. The CO_2 tension of the alveolar air of women and children is from 3 to 5 mm. Hg lower than that of adult males. Due to the compensatory hyperventilation which accompanies the state of acidosis, these values are decreased in acidosis and, due to compensatory hypoventilation, are increased in alkalosis. Within a rather wide range of values, results obtained are in close agreement with those representing the CO_2 combining power of the blood. Mild acidosis is indicated by values ranging from 5 to 3.5 volumes CO_2 per cent (40–25 mm. Hg), moderate to severe acidosis by 3.5 to 2.5 volumes per cent (25–20 mm. Hg) and severe acidosis by values below 2.5 volumes per cent (below 20 mm. Hg). In diabetic coma, values as low as 8 to 10 mm. Hg have been observed. Figures above 6.5 volumes per cent (50 mm. Hg) are indicative of a state of alkalosis. In alkalosis due to primary $\text{CO}_2(\text{H}_2\text{CO}_3)$ deficit in the blood, as is observed in the hyperventilation of high altitudes, hysteria, encephalitis, etc., the CO_2 tension and content of the blood and alveolar air are diminished, a fact which must be borne in mind in the interpretation of results obtained by this method in such conditions.

The Fridericia method of determination of alveolar CO_2 tension yields values which more accurately indicate the CO_2 (H_2CO_3) content of arterial blood, the figures being about 10 per cent lower than those obtained by the Marriott method.

The determination of the CO_2 content and tension of alveolar air, because of its clinical availability and simplicity, is secondary in importance only to the estimation of the CO_2 combining power of the plasma, which, being a more direct index of the state of the alkali reserve of the blood, has to a large extent replaced the indirect procedure in routine practice.

DETERMINATION OF pH OF BLOOD PLASMA OR SERUM

The hydrogen ion concentration of the blood plasma or serum may be determined by colorimetric or electrometric methods. The normal hydrogen ion concentration of blood plasma or serum ranges from pH 7.3 to 7.5, the average value being pH 7.35. The hydrogen ion concentration of the plasma of venous blood is very slightly greater than that of arterial blood, the pH of the former being about 0.03 lower (7.32) than that of the latter. The clinical significance of hydrogen ion concentration determinations is limited because of the fact that abnormal values are obtained only during uncompensated stages of acidosis and alkalosis and therefore no information of positive value is afforded during the earlier, compensated stages, although

0-27 cc. per kilo body weight; mild acidosis (compensated), 27-65 cc. per kilo body weight; moderate to severe acidosis, 65-100 cc. per kilo body weight, severe acidosis, over 100 cc. per kilo body weight. This determination is fairly reliable in indicating the presence or absence of acidosis in diabetes but cannot be utilized as a measure of the degree of acidosis in advanced cases.

Determination of Ketone Bodies. Ketosis, or the accumulation of ketone bodies (aceto-acetic acid, betahydroxybutyric acid and acetone) in the body, is frequently, although not invariably, associated with acidosis. The concentration of ketone bodies in the blood of normal individuals ranges from 1.5 to 2.5 mg. per 100 cc., expressed as acetone. On a mixed diet small quantities of ketone bodies are eliminated in the urine of normal individuals, less than 1 Gm. (expressed as betahydroxybutyric acid) being eliminated in twenty-four hours if sufficient quantities of carbohydrate are present in the diet. Factors which influence the rate of production, accumulation and excretion of ketone bodies are discussed elsewhere (p. 161).

Ketosis and excessive ketonuria are commonly observed in starvation, during periods of carbohydrate privation, in normal pregnancy and in the toxemias of pregnancy, following ether anesthesia, in diabetes mellitus and at times in certain conditions associated with alkalosis, such as hyperventilation, intestinal obstruction and excessive alkali administration. In all of these conditions the fundamental cause of ketosis is probably the same (p. 161). Whether or not acidosis results from ketosis in any given case depends upon the quantity of ketone bodies produced, upon the condition of the alkali reserve and upon the other compensatory mechanisms whereby the body attempts to maintain the normal hydrogen ion concentration of the blood and tissue fluids, namely, hyperventilation, acid elimination in the urine and ammonia formation by the kidney. Acidosis rarely attains a maximum degree of severity as a result of ketosis of normal pregnancy, starvation or carbohydrate privation. It is in diabetes mellitus that the presence of ketosis is most significant, being practically always associated with true acidosis. The concentration of ketone bodies in the blood of patients with diabetic acidosis may reach values of 350 mg. or more per 100 cc. Large quantities of these substances may be eliminated in the urine, a twenty-four-hour output of 50 Gm. or more being not infrequently observed. As stated above, ketonuria is frequently observed in alkalosis due to various causes. In that form dependent upon pyloric or upper intestinal obstruction with continued vomiting, starvation and carbohydrate privation may

nor degree of acidosis should be predicated on the basis of results obtained by this method. This is particularly true of the acidosis of advanced nephritis, in which condition the elimination of the ingested alkali is impaired because of the existing renal functional insufficiency. For this reason the administration of large doses of bicarbonate to individuals with renal failure is not without danger since the state of acidosis may be rapidly converted into one of advanced alkalosis.

Determination of Urinary Ammonia. A normal individual upon an average diet eliminates approximately 0.7 Gm. of ammonia in the urine daily, constituting 2.5 to 4.5 per cent of the total urinary nitrogen. As stated previously, the formation of ammonia in the kidney and its elimination in the urine in combination with acid radicles constitute one of the means whereby the body conserves its supply of available base. The necessity for elimination of increased quantities of acid, as in acidosis, is met by an increase in the quantity of ammonia formed by the kidney, the urinary ammonia being correspondingly increased. An increase in urinary ammonia, therefore, may be indicative of a state of acidosis, a decrease occurring in alkalosis. Values as high as 7 Gm. of ammonia, comprising 50 per cent of the total urinary nitrogen, have been reported in diabetic acidosis.

Advanced grades of acidosis may be present in nephritis (uremia) with no comparable increase in the ammonia content of the urine. This is probably due to the fact that the ammonia-forming function of the kidney has been impaired to such an extent that this compensating mechanism fails to act as it does in the presence of normal renal function. Variations in urinary ammonia may be also produced by dietary factors, the ingestion of acid-forming foods causing an increase and base-forming foods a decrease in the daily output. In advanced hepatic disease urinary ammonia may be increased due to impairment of urea formation by the liver (see p. 419). Under such circumstances the urinary urea is correspondingly decreased.

Titratable Acids in Urine. The ability of the kidneys to excrete increased quantities of acid constitutes one of the mechanisms whereby the body compensates for any tendency toward an increase in the hydrogen ion concentration of the blood plasma. Since this mechanism is closely allied to that of ammonia formation and excretion, the determination of urinary ammonia plus the titratable acid of the urine gives some information as to the state of the acid-base balance. The following figures, expressed in terms of twenty-four hour excretion of $N/10$ acid plus NH_3 , have been given by Van Slyke: normal resting adult,

Chapter XIV

The Respiratory Exchange and Basal Metabolism

THE processes which constitute the phenomenon of respiration are commonly divided into two groups. External respiration is the term applied to the interchange of oxygen and carbon dioxide between the blood and the pulmonary alveoli, internal respiration representing the transportation of these gases in the blood stream and their interchange between the blood and tissues. In order to properly interpret changes which may occur in the oxygen and carbon-dioxide content and tension in the blood and alveolar air, certain facts must be considered regarding the mode of transportation of these gases in the blood stream and their diffusion between the blood and pulmonary alveoli on the one hand and the blood and tissue fluids on the other.

OXYGEN TRANSPORT

Only a small amount of oxygen is carried by the blood in simple physical solution (0.25 to 0.3 volume per cent in arterial blood and 0.1 volume per cent in venous blood). By far the greater part exists in loose combination with hemoglobin, a combination which is remarkable in that it enables the blood not only to abstract from the alveolar air an adequate supply of oxygen but also to permit the diffusion, through the capillaries, of as much as is necessary for tissue oxidation processes.

The volume of oxygen taken up by the blood when it is exposed to atmospheric air, that is, the oxygen capacity, is dependent upon the hemoglobin content of the blood. The maximum amount of oxygen which can combine with 1 Gm. of hemoglobin has not been determined, the commonly quoted value of 1.34 cc. as estimated by Hüfner being probably inaccurate. The matter is one of considerable import since, the oxygen capacity being dependent upon the hemoglobin content, exact knowledge regarding the oxygen capacity of 1 Gm. of hemoglobin would allow the exact determination of the hemoglobin concentration from the oxygen capacity of the blood. It is now recognized that the hemoglobin content of the blood of normal adults may exhibit a diurnal variation amounting to

play an important part in its pathogenesis. The acidosis of nephritis is not consistently associated with ketosis for obvious reasons. Clinically, the presence or absence of this condition is determined usually by the application of qualitative tests for the presence of acetone or diacetic acid in the urine (ketonuria). These qualitative tests serve as roughly quantitative procedures and are satisfactory for clinical purposes. Their chief value lies in the fact that ketonuria is one of the first clinical manifestations of beginning acidosis in diabetes and serves as a valuable therapeutic guide.

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OXYGEN TRANSPORT

Only a small amount of oxygen is carried by the blood in simple physical solution (0.25 to 0.3 volume per cent in arterial blood and 0.1 volume per cent in venous blood). By far the greater part exists in loose combination with hemoglobin, a combination which is remarkable in that it enables the blood not only to abstract from the alveolar air an adequate supply of oxygen but also to permit the diffusion, through the capillaries, of as much as is necessary for tissue oxidation processes.

The volume of oxygen taken up by the blood when it is exposed to atmospheric air, that is, the oxygen capacity, is dependent upon the hemoglobin content of the blood. The maximum amount of oxygen which can combine with 1 Gm. of hemoglobin has not been determined, the commonly quoted value of 1.34 cc. as estimated by Hüfner being probably inaccurate. The matter is one of considerable import since, the oxygen capacity being dependent upon the hemoglobin content, exact knowledge regarding the oxygen capacity of 1 Gm. of hemoglobin would allow the exact determination of the hemoglobin concentration from the oxygen capacity of the blood. It is now recognized that the hemoglobin content of the blood of normal adults may exhibit a diurnal variation amounting to

as much as 20-30 per cent of the average concentration, resulting in similar variations in the oxygen capacity of the blood. The normal range of the latter factor has been estimated to be from 16 to 24 volumes per cent. Haldane and Smith place the average value at 19.5 volumes per cent measured at 0° C. and 760 mm. Hg pressure, more recent determinations by Haden, Osgood and others setting the average oxygen capacity of the blood of normal adult males at about 20.9 volumes per cent. Normal values for

TABLE 6
NORMAL HEMOGLOBIN CONTENT OF HUMAN BLOOD; EFFECTS OF AGE AND SEX
(From Data of Williamson, Hadeo and Appleton)

Age	Oxygen capacity	
	Males, volume per cent	Females, volume per cent
One day.....	32 ± 4	32 ± 4
Two or three days.....	29 ± 4	29 ± 4
Four to eight days.....	26 ± 4	26 ± 4
Nine to thirteen days.....	23 ± 4	23 ± 4
Two to eight weeks.....	20.5 ± 4	20.5 ± 4
Three to five months.....	17.5 ± 3	17.5 ± 3
Six to eleven months.....	16.0 ± 3	16.0 ± 3
One to two years.....	15.5 ± 2	15.5 ± 2
	16.4 ± 2	16.4 ± 2
	17.3 ± 2	17.3 ± 2
	18.0 ± 2	18.0 ± 2
Sixteen to sixty years.....	20.7 ± 2	19.0 ± 2
Sixty to seventy years.....	19.9 ± 2	19.0 ± 2
Seventy years and over.....	19.2 ± 2	18.7 ± 2

After Peters and Van Slyke.

females (adult) are about 1.5 volumes per cent lower than those of males in the age period from sixteen to sixty years. Utilizing Hüfner's factor (1.34), the hemoglobin concentration of the blood of normal adult males would be $\frac{20.9}{1.34} = 15.6$ Gm. of hemoglobin per 100 cc. of blood. As stated above, Hüfner's factor cannot be considered to be accurate, and Williamson, using a spectrophotometric method, has found the hemoglobin concentration to be normally 16.9 Gm. per 100 cc. This value is probably too high. However, utilizing this figure, the oxygen combining capacity of 1 Gm. of hemoglobin per 100 cc. of blood would be $\frac{20.9}{16.9} = 1.235$ cc., a value considerably below that of Hüfner (1.34).

THE RESPIRATORY EXCHANGE AND BASAL METABOLISM

The actual oxygen content of the blood, or the amount of oxygen which combines with hemoglobin, varies with the oxygen tension, the CO_2 tension and the temperature. The CO_2 tension and the temperature remaining constant, an increase or decrease in oxygen tension is associated with a corresponding alteration in hemoglobin saturation with oxygen and hence in the oxygen content of the blood; the oxygen tension and temperature remaining constant, an increase in the CO_2 tension is associated with a decrease in hemoglobin saturation with oxygen.

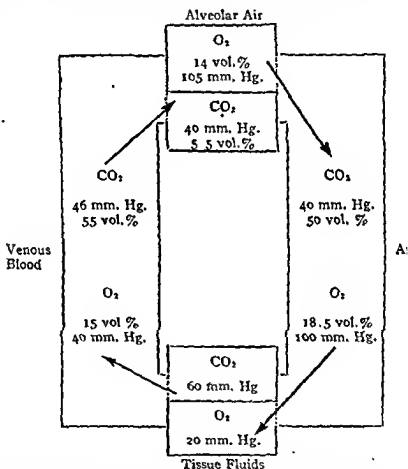


Fig. 12.—Exchange of O_2 and CO_2 between blood, tissue fluids and alveolar air. The partial pressures of O_2 and CO_2 in the alveolar air are 105 mm. Hg. and 40 mm. Hg. respectively. In the venous blood the O_2 tension is 40 mm. Hg. and the CO_2 tension is 46 mm. Hg. In the tissue fluids the O_2 tension is 20 mm. Hg. and the CO_2 tension is 60 mm. Hg. The partial pressures of O_2 and CO_2 in the arterial blood are 100 mm. Hg. and 40 mm. Hg. respectively. The partial pressures of O_2 and CO_2 in the venous blood are 40 mm. Hg. and 46 mm. Hg. respectively. The partial pressures of O_2 and CO_2 in the tissue fluids are 20 mm. Hg. and 60 mm. Hg. respectively. The partial pressures of O_2 and CO_2 in the alveolar air are 105 mm. Hg. and 40 mm. Hg. respectively.

Of particular significance is the fact that the degree of oxygen saturation of hemoglobin does not vary quantitatively directly with alterations in oxygen tension, being relatively much greater at high than at low tensions. For example, with a CO₂ tension of 40 mm. Hg (arterial blood) and an oxygen tension of 100 mm. Hg the degree of hemoglobin saturation may be approximately 98 per cent; with an oxygen tension of 60 mm. Hg the degree of saturation is 90 per cent and at 40 mm. is 76 per cent, a diminution of 22 per cent with a decrease of 60 mm. Hg in oxygen tension. As the tension continues to diminish below 40 mm. Hg the degree of hemoglobin saturation decreases sharply, being about 60 per cent at 30 mm. and 39 per cent at 20 mm., a decrease of 37 per cent in saturation with a drop of 20 mm. in tension. This fact is of particular importance in determining the facility with which oxygen is liberated in actively functioning tissues where the oxygen tension is extremely low.

TABLE 7
EFFECT OF OXYGEN AND CARBON DIOXIDE TENSIONS ON OXYGENATION OF
HEMOGLOBIN IN THE BLOOD OF BOCK
(From Henderson, Bock, Field and Stoddard, J. Biol. Chem. 59: 379, 1924)

O ₂ tension, mm.	Proportion of hemoglobin combined with oxygen at following CO ₂ tensions			
	CO ₂ = 3 mm.	CO ₂ = 20 mm.	CO ₂ = 40 mm.	CO ₂ = 80 mm.
	Per cent	Per cent	Per cent	Per cent
0	0	0	0	0
5	13.5	6.8	5.5	3.0
10	38.0	19.5	15.0	8.0
20	77.6	50.0	39.0	26.0
30	92.0	72.2	60.6	49.8
40	96.7	87.0	76.0	63.5
50	98.5	93.3	85.5	76.9
60	100.0	96.3	90.5	85.0
70	100.0	98.0	94.0	90.3
80	100.0	99.0	96.0	93.7
90	100.0	100.0	97.5	95.7
100	100.0	100.0	98.6	97.1
760	100.0	100.0	100.0	100.0

The degree of oxygen saturation of the blood may be expressed as follows:

$$\frac{\text{Oxygen content}}{\text{Oxygen capacity}} = \frac{18.6}{20.0} = 93 \text{ per cent.}$$

The oxygen content of normal arterial blood has been variously estimated at from 15 to 23 volumes per cent, that of venous

blood being from 10 to 18 volumes per cent, the average value for arterial blood being 18.5 and that for venous blood 15 volumes per cent. The oxygen exchange in the tissues is dependent upon the oxygen content of the blood (oxygen capacity and hemoglobin saturation), the rate and volume of blood flow and the efficiency of the peripheral (capillary) circulation. The coefficient of oxygen utilization by the tissues is an expression of the resultant of these various factors and is determined as follows:

$$\frac{\text{Arterial oxygen} - \text{venous oxygen}}{\text{Arterial oxygen}} = \frac{18.5 - 15}{18.5} = \frac{3.5}{18.5} = 18.9 \text{ per cent.}$$

This figure varies with alterations in the metabolic activity of the tissues as well as with variations in the factors enumerated above.

ANOXEMIA

Anoxemia, meaning oxygen deficiency in the blood, and the more general term anoxia, meaning oxygen deficiency, should properly be applied to any condition of insufficiency of tissue oxidation processes. According to the factors involved, Barcroft has differentiated three types of anoxemia or anoxia and Peters and Van Slyke have added a fourth. These have been termed (1) anoxic anoxia, (2) anemic anoxia, (3) stagnant anoxia and (4) histotoxic anoxia.

Anoxic Anoxia. This group includes conditions characterized by normal oxygen capacity but diminished oxygen tension in the arterial blood with a consequent varying degree of hemoglobin unsaturation.

(a) *High Altitudes.* The condition commonly designated mountain sickness is in reality a state of anoxic anoxemia due to diminished oxygen tension in the atmospheric air and consequently in the alveolar air and blood stream

(b) *Rapid, Shallow Respiration.* Shallow breathing is conducive to inefficient oxygenation of the blood since, if the volume of tidal air is greatly decreased, comparatively little fresh air passes the physiologic dead space (150 cc.) to enter the alveoli. It is questionable whether this factor, in itself, is capable of producing anoxemia in disease states but it is unquestionably a contributory factor in such conditions as pneumonia, in which anoxemia is dependent largely upon other factors.

(c) *Mechanical Interference with Oxygen Absorption.* This condition exists in pneumonia, pulmonary edema, pulmonary congestion, emphysema, bronchial asthma, acute bronchitis and other diseases of the respiratory tract. This type of anoxic anoxemia is the one most commonly observed clinically. Obviously,

any condition which interferes with the passage of atmospheric air into the pulmonary alveoli or with the diffusion of oxygen from the alveoli into the blood will result in diminution in the oxygen content of the blood. It must be recognized, however, that a considerable portion of the ventilating surface of the lung may be functionally incapacitated with no alteration in oxygenation of the blood. Whether or not anoxemia occurs in these conditions is determined largely by (1) the circulation of blood in the affected area and (2) the rate of blood flow through the unaffected portions of the lung. In conditions such as unilateral pneumothorax and pleural effusion, the lung of the opposite side being normal, there is practically no circulation of blood in the collapsed lung. Since practically all of the blood returning to the heart in the pulmonary vein has passed through the normal lung and has consequently been adequately oxygenated, anoxemia does not occur. Similarly, in the early stages of lobar pneumonia, when the anatomic change consists of complete consolidation of a portion of the lung, anoxemia does not exist since little or no blood has circulated through the consolidated area. In bronchopneumonia, however, and in lobar pneumonia with an advancing lesion of a bronchial type, the circulation of blood through poorly aerated alveolar areas is relatively unimpaired and therefore the blood which returns to the heart in the pulmonary vein is partly oxygenated and partly nonoxygenated, cyanosis being a common manifestation under such circumstances. This anoxemic tendency is further aggravated by the frequently coexisting bronchitis and pulmonary edema. Furthermore, even in conditions such as pure lobar pneumonia, the rate of blood flow through the well aerated portions of the lung may be so increased that deficient oxygenation occurs as a result of the relatively brief exposure of the hemoglobin to alveolar oxygen. The degree of anoxemia has a distinct bearing upon prognosis in lobar pneumonia. Stadie found that in sixty-one fatal cases the degree of hemoglobin unsaturation varied from 14 to 52 per cent, the average being 32 per cent, a tremendous increase over the normal unsaturation value of 5 per cent. Fifteen of sixty patients who recovered at no time showed more than 16 per cent of arterial hemoglobin to be in the unsaturated form, a figure of 33 per cent being obtained at one time in one case.

(d) *Congenital Heart Disease.* In certain cases of congenital cardiac septal defects a portion of the blood may flow directly from the right to the left side of the heart without having passed through the lungs, the mixture of aerated and nonaerated blood in the systemic circulation resulting in a state of anoxic anoxemia. The condition is apparently further aggravated by incom-

plete oxygenation of the blood that does flow through the lungs. Anoxic anoxemia is relatively infrequently observed in congenital heart lesions associated with septal defects because the pathologic intracardiac deviation of blood flow is usually from the left to the right rather than from right to left.

The characteristic chemical feature of this type of anoxemia is an abnormally low oxygen saturation of the hemoglobin of arterial blood. Because of the compensatory polycythemia which usually occurs, particularly if the underlying condition is of a chronic nature, the oxygen content of arterial blood may be within normal limits or even actually increased. The oxygen content of venous blood may be normal or diminished depending upon the arterial blood values and upon the degree of oxygen utilization in the tissues.

Anemic Anoxia. This type of anoxemia is characterized by a diminution in the oxygen capacity of arterial blood due to a decrease in the amount of functioning hemoglobin.

(a) *Anemia.* The occurrence of anoxemia in anemia is readily understandable, the degree of oxygen saturation of arterial blood being normal but its oxygen content being diminished in proportion to the decrease in hemoglobin.

(b) *Carbon Monoxide Poisoning* (p. 99). Carbon monoxide combines with the same group in the hemoglobin molecule as does oxygen, the combining capacity of hemoglobin for both gases being identical. However, the affinity of hemoglobin for carbon monoxide is more than 200 times as great as its affinity for oxygen, and therefore in the presence of relatively small concentrations of carbon monoxide in the air a considerable quantity is taken up by the blood with a consequent reduction in the amount of hemoglobin available for the transportation of oxygen to the tissues. Furthermore, the presence of carbon monoxide in the blood apparently diminishes the facility of dissociation of oxyhemoglobin and therefore increases the existing anoxemic tendency by interfering with the liberation of oxygen from the blood in the tissues.

(c) *Methemoglobinemia* (p. 98). Methemoglobin is a substance, formed from reduced hemoglobin through the action of an oxidizing agent, which has the peculiar property of not being capable of combining with oxygen. Anoxemia associated with methemoglobinemia is of the anemic type inasmuch as it is due to a decrease in the quantity of functioning hemoglobin in the circulating blood.

(d) *Sulfhemoglobinemia* (p. 99).

Stagnant Anoxemia. Stagnant anoxia is due to circulatory inefficiency, the rate of blood flow through the tissues being

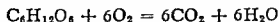
retarded with resulting increase in the percentage volume of oxygen removed from the blood in its passage through the capillaries. It is observed most commonly in circulatory failure associated with decompensated heart disease, in shock and in conditions associated with vasospastic phenomena such as Raynaud's disease. In this type of anoxia the arterial oxygen tension, capacity and content may be normal, but because of the excessive oxygen loss in the tissues the venous oxygen tension and content are subnormal. In myocardial failure, anoxic anoxia is superimposed upon stagnant anoxia because of the impaired diffusion of oxygen from the alveolar air into the blood through the congested pulmonary alveoli.

Histotoxic Anoxia. Histotoxic anoxia is a term suggested by Peters and Van Slyke to indicate a condition in which the oxygen supply is normal in every respect but the degree of oxygen utilization by the tissues is diminished because the tissue cells are poisoned in such a manner that they cannot use oxygen properly. Histotoxic anoxia occurs in poisoning by alcohol, cyanide, and perhaps formaldehyde and acetone. In the absence of complicating factors such as shock, the arterial and venous oxygen tension, capacity and content are within normal limits.

RESPIRATORY QUOTIENT

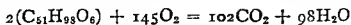
The "respiratory quotient" is a term applied by Pflüger to the ratio of the volume of carbon dioxide expired to the volume of oxygen inspired during the same interval of time. The value of the respiratory quotient depends upon the nature of the food-stuffs metabolized, the determining factor being their relative content of hydrogen and oxygen.

The carbohydrate molecule contains hydrogen and oxygen in the proportion to form water and therefore the complete oxidation of carbohydrates, as typified by that of glucose, may be expressed as follows:



It is obvious from this equation that 1 volume of oxygen absorbed produces 1 volume of carbon dioxide, the respiratory quotient for carbohydrate being therefore 1.00.

Fats contain relatively more hydrogen in proportion to their oxygen content and therefore, during the process of combustion, more oxygen is required, not only for the production of carbon dioxide but also for the oxidation of hydrogen to water. The oxidation of fats, as typified by tripalmitin, may be illustrated by the following equation:



The respiratory quotient for fat is therefore

$$\frac{102 \text{ volumes CO}_2}{145 \text{ volumes O}_2} = 0.707$$

The respiratory quotient for protein is dependent upon the oxidation of the nonnitrogenous portion or the carbohydrate moiety of its various constituent amino acids. The respiratory quotient for meat protein has been calculated to be 0.802.

If the urinary nitrogen and the respiratory quotient are known, the substances oxidized in the organism may be determined. It has been found that for every gram of nitrogen in the urine derived from protein, 8.49 Gm. of oxygen have been inspired and 9.35 Gm. of CO₂ have been eliminated. On the basis of these figures the nonprotein respiratory quotient may be estimated by subtracting from the total CO₂ elimination that portion derived from protein (Gm. urinary nitrogen \times 9.35) and from the total oxygen intake the proportion required to oxidize protein (Gm. urinary nitrogen \times 8.49). The nonprotein respiratory quotient is 0.707 when fat alone is being oxidized and 1.00 when carbohydrate alone is being oxidized, values between these two figures indicating the combustion of mixtures of carbohydrate and fat. On the basis of the nitrogen elimination in the urine and the volume of oxygen utilized and carbon dioxide eliminated one may calculate exactly the amounts of protein, carbohydrate and fat oxidized during the experimental period. Tables are available by means of which the relative percentages of fat and carbohydrate oxidized may be readily determined if the nonprotein respiratory quotient is known.

Under certain circumstances the respiratory quotient may rise considerably above 1.00. When carbohydrates, which are rich in oxygen, are converted into fats, which are poor in oxygen, the volume of oxygen inspired may be relatively much less than the volume of carbon dioxide eliminated, the respiratory quotient being consequently increased. Values above 1.3 may be observed under such circumstances. Then, too, if carbohydrate is supplied in abundance during periods of prolonged strenuous exercise, the respiratory quotient may rise due to the liberation of CO₂ from NaHCO₃ by the increased amounts of lactic acid formed as a result of the excessive muscular exertion. Extremely low respiratory quotient values (0.6 or less) may be obtained during periods of conversion of fat to carbohydrate in the organism since the transformation of an oxygen-poor substance into an oxygen-rich substance is necessarily associated with a relative increase in the volume of inspired oxygen as compared with that of expired carbon dioxide. Respiratory

quotient values under different metabolic conditions are as follows:

Purely carbohydrate diet.....	1.0
Purely protein diet.....	0.8
Purely fat diet.....	0.7
Mixed diets.....	0.85
Conversion of fats to carbohydrates.....	0.7-(0.6)
Conversion of carbohydrates to fats.....	1.0 + (1.3)
Sixteen hours after meals.....	0.82

The determination of the respiratory quotient is of particular value in studying the severity of the metabolic error in diabetes mellitus since it affords an accurate index of the degree of impairment of carbohydrate utilization. With increasing severity of the condition the respiratory quotient values decrease from those observed under resting conditions in normal individuals on a mixed diet (0.85), approaching 0.7 in complete diabetes, indicating the predominant role played by fat oxidation in the maintenance of the metabolic requirement of the diabetic organism. The administration of insulin is followed by an increase in the respiratory quotient in both normal individuals and those with diabetes. Following the administration of glucose to normal individuals the respiratory quotient may rise from 0.82 to 0.96 or more in about two hours whereas in individuals with diabetes there may not only be no rise in the respiratory quotient but in some cases an actual fall occurs which may persist for several hours. Similarly, other factors which normally increase the respiratory quotient, such as muscular exercise and the administration of epinephrine, have much less effect in this regard in diabetic patients.

Some authorities believe that overproduction of glucose (from protein and fat) rather than impaired utilization of glucose constitutes the fundamental underlying abnormality of carbohydrate metabolism in diabetes mellitus. The R.Q. for the conversion of protein to glucose has been estimated as 0.632 to 0.706, that for gluconeogenesis from fat being about 0.281. According to the proponents of the overproduction theory, the usual diabetic R.Q. of 0.7 is regarded as the resultant of two processes proceeding simultaneously: (a) gluconeogenesis from protein and fat, with an R.Q. ranging from 0.2 to 0.7, and (b) oxidation of carbohydrate, with an R.Q. of 1.0.

The basal respiratory quotient in individuals with hyperthyroidism is usually subnormal, owing probably to the depleted state of the glycogen reserve in the tissues which occurs as a result of the increased rate of carbohydrate utilization manifested in that condition. Following the administration of glucose the respiratory quotient usually rises more rapidly and to a

higher degree than in normal individuals, with a more rapid return to the basal level, an observation which indicates an increased rate of carbohydrate utilization in hyperthyroidism.

BASAL METABOLISM

Basal metabolism, otherwise termed "standard metabolism" or postabsorptive metabolism, is an expression used to designate the energy (heat) output of the body at complete mental and physical rest (twelve to sixteen hours after the last meal). Under these resting conditions the heat production of different organs varies considerably, approximately 25 per cent of the basal energy output (basal metabolism) being due to the functional activity of the kidneys, heart and liver and the respiratory movements, the remaining 75 per cent representing the heat production of resting tissues such as skeletal muscle. Since metabolic processes are essentially oxidative in nature, involving the utilization of oxygen, the liberation of carbon dioxide and the production of energy in the form of heat, basal metabolism may be expressed in terms of any one of the three factors involved; *i.e.*, (1) calories produced, (2) oxygen utilized or (3) carbon dioxide liberated. As is true of many physiologic processes, it has been found that heat production is more directly proportional to the surface area of the body than to any other single factor and it has therefore been found convenient to utilize this factor as a unit of measurement. Accordingly, the basal metabolic rate is expressed in terms of calories per square meter of body surface per hour.

The basal metabolic rate may be determined by direct or by indirect methods. The direct method, which consists in placing the individual to be examined in a calorimeter and actually measuring the amount of heat produced in a given time, is unquestionably the most accurate method of determining basal metabolism. However, the extreme complexity and great expense of the apparatus render it unavailable and impracticable for clinical purposes. The indirect methods, in their present state of perfection, are extremely reliable and possess the distinct advantages of simplicity of operation and a high degree of accuracy. Basal metabolism may be determined indirectly in two ways, the so-called "open or gasometric method" being more commonly employed abroad and the closed or spirometric method being most popular in this country.

Open or Gasometric Method. The patient breathes atmospheric air through a specially constructed mouthpiece over a definite period of time, the expired air for the same period being collected in a Douglas bag. The total volume of expired air is

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Closed or Spirometric Method. This method consists in the direct volumetric determination of the quantity of oxygen removed from an oxygen chamber within a definite period of time. Various forms of apparatus have been devised, the details of which cannot be entered into here. This method is very widely used in this country since the type of apparatus now available combines accuracy with ease of operation, no chemical analyses being necessary. The oxygen consumption is determined directly in terms of cubic centimeters per minute and the subsequent calculation of the basal metabolic rate may be made in one or both of the two ways described above, *i.e.*, on the basis of either oxygen consumption or heat production.

CALCULATION OF BASAL METABOLIC RATE

Since the available tables of normal values for oxygen consumption and heat production have been based upon observations upon males between twenty and fifty years of age, corrections must necessarily be made for age and sex in the case of females and individuals below twenty and above fifty years of age.

Males

- 16-18... add 7 per cent to value in table
- 18-20... add 5 per cent to value in table
- 50-60... subtract 4 per cent from value in table
- 60-70... subtract 7 per cent from value in table

In the case of *females*, subtract 7 per cent from the normal value for a male of the same age, height and weight; in the opinion of the Nutrition Laboratory of the Carnegie Institute of Washington the normal standards for women are a little too high and should be lowered.

The basal metabolic rate is expressed in terms of percentage above or below the normal oxygen consumption or heat production for an individual of the same sex, age, height and weight; the following examples illustrate the manner in which the actual calculations are made.

Calculation on Basis of Oxygen Consumption per Minute.

Female, age 19, height 58 in., weight 117 lbs.

Actual oxygen consumption: 210 cc. per minute.

Normal oxygen consumption for adult male: 188 cc.

Correction for age: $188 \times 1.05 = 197.4$ cc.

Correction for sex: $197.4 \times 0.93 = 183.58$ cc.

$$\text{B. M. R.} = \frac{210 - 183.58}{183.58} \times 100 = +14.3 \text{ per cent.}$$

ascertained by means of a gas meter and its oxygen and carbon dioxide contents are determined by gas analysis in a Haldane or Van Slyke apparatus. The difference between the oxygen and carbon dioxide contents of atmospheric and expired air represents the amount of oxygen utilized and carbon dioxide eliminated. Corrections must be made for water vapor tension, barometric pressure and temperature. The respiratory quotient is calculated from these data. The basal metabolic rate may then be calculated in one of three ways.

(a) The caloric value of oxygen inspired varies according to the proportions of protein, carbohydrate and fat which have been oxidized during the experimental period. The caloric value of one liter of oxygen for each of the foodstuffs is as follows:

	Calories
Protein (respiratory quotient 0.802).....	4.463
Fats (respiratory quotient 0.707).....	4.686
Starch (respiratory quotient 1.00).....	5.047
Sixteen hours after meal (respiratory quotient 0.82).....	4.8

Tables are available which indicate the caloric values of one liter of oxygen at different respiratory quotient levels. Having determined the respiratory quotient and the volume of oxygen utilized over a given period of time the heat production of the patient per hour may be readily determined. The surface area is obtained from the height and weight by referring to tables based upon the height-weight formula of DuBois; the basal metabolic rate is then expressed in terms of calories per square meter of body surface per hour.

(b) The procedure may be simplified by dispensing with the determination of the CO_2 elimination and respiratory quotient, measuring only the oxygen inspired. It is assumed that tissue metabolism sixteen hours after the last meal is such that the respiratory quotient is 0.82 and the caloric value of one liter of oxygen is 4.8 calories. This assumption has been found to be sufficiently accurate for clinical purposes, rarely involving an error of more than 1-2 per cent. In many cases, particularly in untrained subjects, the determination of the respiratory quotient involves an error which is greater than this figure. Knowing the height, weight and oxygen consumption per hour, the basal metabolic rate may then be expressed as above in terms of calories per square meter of body surface per hour.

(c) The procedure may be still further simplified by basing the calculation entirely upon the oxygen consumption in terms of cubic centimeters of oxygen utilized per minute. This figure may then be compared with the known normal values for oxygen consumption of an individual of the same height and weight, as indicated in available tables.

INCREASED BASAL METABOLIC RATE

Hyperthyroidism. Increase in the basal metabolic rate is perhaps the most characteristic manifestation of hyperthyroidism, the degree of rise paralleling the severity of the condition. Hyperthyroidism may occur without any apparent gross anatomic change in the thyroid gland, or in association with exophthalmic goiter, adenoma of the thyroid or, less commonly, malignancy or acute inflammatory processes in the thyroid gland. The active principle of thyroid secretion is thyroxin, a remarkably active substance which, as stated by Plummer, may be regarded as a catalyst that accelerates the formation of a quantum of potential energy in the cells of the organism. As stated by Lusk, "a milligram of thyroxin, the most powerful agent influencing metabolism, may be responsible for an increase in the heat production equal to the oxidation of 267 Gm. of glucose or 267,000 times the weight of the catalyst. This has been defined as the calorogenic power of thyroxin." The action of thyroxin or thyroid extract in the body is prolonged; following their administration, metabolism rises to a maximum in from six to eight days and then gradually declines, reaching the resting level after a variable period of time depending upon the amount of the substance administered. For example, in a patient reported by Boothby, suffering with myxedema with a basal metabolic rate of minus 30, following the intravenous injection of 24 mg. of thyroxin the basal metabolic rate rose to a maximum of plus 18 on the tenth day and then gradually declined, being minus 15 on the forty-fourth day and minus 20 on the fifty-second day.

The basal metabolic rate probably affords the best single index of the severity of the disease (hyperthyroidism) and is invaluable for determining the operative risk in any case. Values above plus 100 per cent have been observed, cases showing values of plus 20 to plus 50 being considered as mild or moderately severe, plus 50 to plus 75 severe, and above plus 75 very severe. Thus, determination of the basal metabolic rate is valuable from the standpoints of diagnosis, prognosis and the determination of operability in any given case.

Other Endocrine Disorders. Elevated values (plus 20 to plus 35) are occasionally found during relatively brief periods in the early stages of acromegaly. This increase in the basal metabolic rate is probably due to hypersecretion of the pituitary gland, a condition which exists for but a relatively short period in the life history of acromegaly, being rapidly superseded by hypopituitarism with consequent diminution in the basal meta-

Calculation on Basis of Heat Production.

Male, age 35, height 67 in., weight 154 lbs. Surface area 1.8 square meters.

Actual oxygen consumption: 200 cc. per minute, or 12 liters per hour.

Heat expenditure: $12 \times 4.825 = 57.9$ calories per hour.

Calories per square meter: $\frac{57.9}{1.8} = 32.16$ calories.

Average normal (DuBois): 39.5 calories per hour per square meter.

$$B. M. R. = \frac{39.5 - 32.16}{39.5} \times 100 = -18.5 \text{ per cent.}$$

As stated by Barach and Draper, the value of the determination of basal metabolism in clinical practice appears to lie largely in the exclusion of the thyroid gland as the cause of the patient's symptoms. A large volume of accumulated data indicates that about 75 per cent of individuals without thyroid disease have a basal metabolic rate ranging from plus 10 to minus 10 per cent whereas 95 per cent of such individuals fall within the limits of plus 15 to minus 15 per cent (DuBois standards). It is probable therefore, that the latter figures (plus 15 to minus 15) must be regarded as the range for all individuals not suffering with thyroid disease, although the narrower limits (plus 10 to minus 10) perhaps more accurately represent the strictly normal range. The basal metabolic rates of normal individuals under strictly basal conditions are remarkably constant. During the performance of the test, however, care must be exercised to avoid such conditions as nervousness, emotional excitement, movements of the arms and legs, uncomfortable positions, distention of the bladder and even conversation, all of which may cause some rise in metabolism, particularly in neurotic individuals. There is also evidence to suggest that individuals who have previously been on a high caloric diet, particularly if the protein-content is high, exhibit basal metabolic rates distinctly above those of individuals on a low caloric, low protein intake. Extremely high and extremely low external temperatures may also cause a rise in the basal metabolic rate. Season, time of day and sunlight are apparently without distinct effect. Some observers have reported lower values for Orientals than for Occidentals but these findings have not been supported by recent observations. Certain drugs, particularly caffeine, to a lesser degree atropine, increase the metabolic rate which is of course distinctly raised in normal individuals following the administration of thyroid extract, pituitrin or epinephrine. The metabolic rate may be slightly diminished following the administration of morphine.

be an important factor, as indicated by the high blood uric acid concentration in many cases, particularly in chronic myelogenous leukemia.

High values ranging from 16 to 40 per cent may be observed in true polycythemia or erythremia, although in some cases the basal metabolic rate may be within normal limits. It has been suggested by Isaacs that the increase may be due to the excessive destruction of nuclear material as evidenced by increased uric acid production.

Anemia, particularly pernicious anemia and some cases of splenic anemia, may be associated with an increased metabolic rate, the cause of which is unknown. It is probably due to the underlying condition rather than to the anemia itself. In some cases of severe secondary anemia the basal metabolic rate may be increased.

Essential Hypertension. In the great majority of cases of essential hypertension the basal metabolic rate is within the normal limits of plus 15 to minus 15 per cent. However, in some instances values as high as plus 70 per cent may be observed, figures above plus 20 per cent having been obtained in 3.4 per cent of a series of 170 cases studied by Boothby and Sandiford. The cause of the increased metabolic rate in these cases in the absence of myocardial insufficiency and dyspnea is not clear. Some observers have attributed it to increased thyroid secretion and others to increased adrenal secretion.

Myocardial Insufficiency. Heart disease in itself has apparently no effect upon the metabolic rate. However, values as high as plus 40 per cent may be obtained in patients with myocardial failure. This is due partly to the increased activity of the respiratory muscles incident to the dyspnea associated with heart failure and partly to the increased oxygen consumption which results from slowing of the circulation in the tissues.

Diabetes Insipidus. Diabetes insipidus may be associated with an increase in the basal metabolic rate amounting in some instances to as much as 30 to 45 per cent. The explanation for this phenomenon is not clear, perhaps the most probable hypothesis being that it is dependent upon the enormous increase in the work of the kidneys required for the elimination of the excessive quantities of water. Diminution in the renal output following the administration of pituitrin is followed by a return of the basal metabolic rate to a normal level.

DECREASED BASAL METABOLIC RATE

Hypothyroidism. Cretinism, myxedema and minor grades of hypothyroidism are associated with a decreased basal metabolic

bolic rate. In the great majority of cases the reported values are well within normal limits. Increased values may also be found in patients with basophilic adenoma of the pituitary gland, the condition known as Cushing's syndrome.

The administration of adrenalin is rapidly followed by an increase in the metabolic rate. Clinical states of hyperadrenalism are occasionally observed during certain stages of the growth of tumors of the adrenal gland, such as pheochromocytomas, and may be associated with an increase in the basal metabolic rate. It is believed by some that this action of epinephrine is due not to a specific calorogenic effect as in the case of thyroxin, but rather to the generalized increase in muscle tonus which results from sympathetic stimulation.

Pregnancy and Lactation. The basal metabolic rate is rather consistently increased during the later months of pregnancy and particularly during lactation. It is probable that the increased metabolism in pregnancy is due entirely to the metabolism of the fetus and that the metabolic rate is proportional to the combined surface area of mother and fetus. During lactation, however, high values are frequently observed which may perhaps be ascribed to the increased activity of the mammary glands.

Fever. An increase in the basal metabolic rate occurs in practically all fevers, the average rise being about 13 per cent for each degree Centigrade or 7.2 per cent for each degree Fahrenheit. A relatively greater rise occurs in cases in which toxemia is increased out of proportion to the fever, the destruction of body protein in such instances being increased. In tuberculosis, on the other hand, the rise in the basal metabolic rate is relatively less than in other febrile disorders, due to the fact that in that condition the toxemia is usually comparatively slight and there is relatively little toxic destruction of body protein. The malnutrition which is commonly associated with tuberculosis may also be a factor in the production of this tendency toward a relatively low metabolic rate.

Diseases of the Blood. The basal metabolism is increased in the great majority of patients with both lymphatic and myelogenous leukemia even in the absence of fever, the rise being in many instances comparable to that observed in hyperthyroidism. The exact cause of this phenomenon is not known; the basal metabolic rate does not appear to parallel the total leukocyte count but may be dependent rather upon the increased activity of the bone marrow or lymphatic apparatus which may not be accurately indicated by the number of immature cells in the circulating blood. An increased rate of protein metabolism may

and nephritis, who are frequently malnourished, may show similar findings. Subnormal values, at times as low as minus 40 per cent, have been reported in patients with anorexia nervosa, a condition which may be difficult to distinguish from pituitary cachexia (Simmonds' disease). The low basal metabolic rate in anorexia nervosa is probably due largely to inanition.

Miscellaneous. Subnormal metabolic rates may be obtained at times in patients with severe anemia, chronic arthritis, peptic ulcer, and a variety of diseases of the nervous system, including psychoneurosis, schizophrenia and autonomic imbalance (vagotonia).

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rate, the extent of the decrease being an accurate indication of the degree of thyroid functional insufficiency. Values below minus 35 per cent are uncommon although figures as low as minus 42 per cent have been observed in severe cases of myxedema. The statement made with regard to the value of the determination of the basal metabolic rate in hyperthyroidism apply equally to hypothyroidism, its determination being almost essential for the diagnosis of mild grades of hypothyroidism and in following the response to therapy in severe cases.

Other Endocrine Disorders. Hypoadrenalism, either functional or associated with organic disease of the adrenal glands such as tuberculosis (Addison's disease), may be associated with a decrease in the basal metabolic rate, usually not, however, to the degree observed in hypothyroidism. Values below minus 25 per cent are extremely unusual. Similar findings may be obtained in hypopituitarism, which may occur either as a congenital disturbance or as a manifestation of the destructive action of tumors of pituitary gland. Very low figures, at times below minus 40 per cent, are characteristically obtained in patients with pituitary cachexia (Simmonds' disease). This condition is characterized primarily by marked or complete atrophy of the hypophysis with extreme hypofunction and at times atrophy of the entire endocrine system.

The Nephrotic Syndrome. The basal metabolic rate is usually subnormal in patients with "lipoid nephrosis," being frequently about minus 20 per cent and occasionally as low as minus 35 per cent. These low values are most commonly obtained during the periods in which edema is marked but cannot be explained satisfactorily solely upon the basis of the edema in many cases. Epstein believes that "lipoid nephrosis" may be primarily a disturbance of protein metabolism with subnormal thyroid function and has found that patients suffering with this disease exhibit a markedly increased tolerance to thyroid extract, the administration of which is followed by strikingly beneficial effects in some instances. However, there is little evidence to support this view, which has not met with general acceptance.

Shock. The basal metabolism may be strikingly diminished in shock, the degree of diminution being of some value from the standpoint of prognosis.

Malnutrition. The basal metabolic rate may be low in conditions associated with malnutrition or starvation. Protein privation appears to be the most important factor in these cases. Normal individuals receiving a low caloric diet deficient in protein may exhibit basal metabolic rates as low as minus 25 to minus 30 per cent. Similarly, individuals with diabetes mellitus

"saturation tests," although results obtained by these procedures are perhaps not subject to as rapid fluctuation as the blood values. The extent of the tissue changes, indicating the severity of the deficiency state, can be best determined, of course, by biomicroscopy. In certain instances, however, rather prolonged deficiency is required for the production of morphologic abnormalities, and blood or urine studies or saturation tests may yield earlier abnormal results. In established chronic deficiency states, which are the type encountered most frequently clinically, blood and urine studies may be misleading, particularly during periods of specific therapy.

VITAMIN A^{6,10,11,27,30}

There is little substantial evidence that vitamin A deficiency is accompanied by any significant disturbance of protein, fat or carbohydrate metabolism. There have been isolated reports of marked increase in the esterase content of the blood serum in vitamin A deficient rats¹ and of an increase in serum cholesterol following administration of excessive amounts of vitamin A. In the growing dog with vitamin A deficiency there is increased osteoblastic and osteoclastic activity, with proliferation of cancellous bone at the expense of compact bone, the overgrowth of bone causing compression of adjacent nerve fibers and cells, with consequent changes in the central nervous system.^{25,35} This factor may also be necessary for the normal development of the teeth.^{9,33}

Vitamin A and provitamin A, carotene, are absorbed from the intestine, bile salts being necessary for absorption of the latter. Absorption of vitamin A is impaired in the absence of pancreatic enzymes (impaired hydrolysis of vitamin A esters) and in celiac disease or other conditions in which there is impaired absorption of dietary fat (e.g., obstructive jaundice, chronic pancreatitis).^{24,36} The liver is apparently the chief site of conversion of carotene to vitamin A as well as the chief site of storage of the latter, which is mobilized in response to physiologic demands. Ingestion of alcohol,^{5,12} sympathico-adrenal stimulation³⁷ or administration of certain carcinogenic polycyclic hydrocarbons (e.g., benzanthracene)⁴ result in active mobilization of vitamin A from the liver, with a rise in its concentration in the blood. The quantity of vitamin A in the liver has been found to be low in acute infections, cirrhosis and vitamin A deprivation.^{2,18,32}

The normal concentrations of vitamin A and carotene in the blood plasma have been given by some as 100-300 (average 200) I.U. of vitamin A and 60-260 (average 145) micrograms of

Chapter XV

Vitamins

CERTAIN aspects of vitamin deficiency fall within the scope of biochemical investigation. Much of the data pertinent to this subject has been considered elsewhere, but it seems desirable to assemble the available information here in summary form. Various types of laboratory study may aid in the detection of certain vitamin deficiency states. They include biochemical, microbiologic, biophysical and biomicroscopic studies,²¹ applied as follows:

(a) Biochemical and microbiologic (growth of micro-organisms) methods are employed for the determination of the concentration of certain of the vitamins in the blood, urine and other body fluids.

(b) The curve of concentration in the blood or excretion in the urine may be measured after administration of a standard test dose of the vitamin (saturation tests).

(c) Quantitative determinations may be made of the vitamin content of tissues obtained at biopsy, or evidence of deficiency may be obtained by microscopic examination of tissues (*e.g.*, mucosal scrapings in vitamin A deficiency).

(d) Certain consequences of deficiency may be demonstrated by biophysical methods, *e.g.*, impaired dark adaptation in vitamin A deficiency and increased capillary fragility in vitamin C deficiency.

We are concerned here chiefly with quantitative estimations of the vitamins or their metabolic products in the body fluids. Such methods are available at present for only a few of the known vitamins. Moreover, they have distinct limitations in their applicability to the appraisal of nutritional status. Inasmuch as the blood is a labile transport medium, its vitamin content in chronic deficiency states may be increased after a comparatively brief period of high vitamin intake without comparable improvement in the morphologic abnormality in the tissues. As stated by Kruse,²¹ in the evolution and recession of an avitaminosis, changes in concentration of the vitamin in the blood and urine do not occur synchronously with alteration in the tissue state. The same may be said of the so-called

"saturation tests," although results obtained by these procedures are perhaps not subject to as rapid fluctuation as the blood values. The extent of the tissue changes, indicating the severity of the deficiency state, can be best determined, of course, by biomicroscopy. In certain instances, however, rather prolonged deficiency is required for the production of morphologic abnormalities, and blood or urine studies or saturation tests may yield earlier abnormal results. In established chronic deficiency states, which are the type encountered most frequently clinically, blood and urine studies may be misleading, particularly during periods of specific therapy.

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ingestion of carotene in oil is higher and the rise more prolonged in diabetics than in normal subjects.^{17,21} It was suggested that this may be due to impaired formation of vitamin A from carotene (hepatic dysfunction). These findings have been contradicted by other workers.²²

Carotinemia may be confused with hyperbilirubinemia if reliance is placed on the icterus index determination alone (p. 436), which is elevated in this condition. The serum bilirubin concentration is normal in uncomplicated carotinemia and the diagnosis is established by determination of the serum carotenoid (carotene) concentration.

VITAMIN B₁ (THIAMINE)^{5a,11a,24}

Vitamin B₁ (thiamine) exerts an important influence upon metabolic processes.^{1,4,5} Interest has been centered particularly upon its influence on carbohydrate metabolism. Certain of the more important observations in this connection have been presented elsewhere (p. 16). They may be summarized as follows: In the absence of adequate amounts of vitamin B₁, certain parts of the brain (also kidney and heart muscle) show a diminished oxygen uptake in the presence of dextrose and an increase in pyruvic acid in the presence of lactic acid, both of these abnormalities being corrected by addition of the vitamin. It has been concluded, therefore, that vitamin B₁ acts to bring about oxidation of pyruvic acid. In confirmation of these observations, significant increases in bisulfite-binding substances (chiefly pyruvic acid) have been found in the blood, urine and cerebrospinal fluid of patients and animals in a state of vitamin B₁ deficiency (beri-beri).⁵ The most widely accepted hypothesis is that this vitamin acts as a catalyst co-enzyme in the metabolism of carbohydrate, more specifically in the breakdown of pyruvic acid. It is believed that this function is probably not merely to aid in the degradation of pyruvic acid but to utilize it in some metabolic process. It appears probable that the active vitamin is a pyrophosphoric ester of thiamine, formed in the body by phosphorylation of the latter. This factor appears to act as a cocarboxylase; *i.e.*, in association with a protein present in yeast cells, it promotes the evolution of CO₂ from pyruvic acid, with the formation of acetaldehyde, a reaction which does not involve oxidation. This cocarboxylase, like free thiamine, also increases the oxygen uptake of polyneuritic tissue (catatorulin effect).⁶ It has also been suggested that the demonstrated rôle of this vitamin in carbohydrate metabolism is merely one manifestation of its more fundamental significance in biological oxidation reactions. The hypothesis has also been

carotene per 100 cc.,^{14,20} and by others as 20-43 micrograms of vitamin A and 100-368 micrograms of carotene per 100 cc. Neither vitamin A nor carotene is excreted in the urine of human beings under normal conditions unless large amounts have been administered, being apparently readily destroyed or stored in the organism. Both are excreted in human colostrum and milk.

The plasma vitamin A concentration is usually but not invariably subnormal in subjects maintained on a diet deficient in vitamin A, and, although there may be a general parallelism between the intake and the concentration in the blood, the value of blood vitamin A values in assessing nutritional status in this connection is uncertain.²³ Subnormal plasma values have been obtained also in conditions mentioned above in which absorption from the intestine is impaired, in gastro-intestinal malignancy¹ and in a number of types of hepatic disease. No definite correlation has been established between the results of biophotometric studies and the vitamin A content of the blood, which apparently bears no constant relation to the adequacy of the vitamin stores in the body. However, the statement has been made, on the basis of an extensive study of this problem, that a high value for vitamin A in the blood is inconsistent with deficiency of this vitamin.¹⁹ Low values for vitamin A and carotene have been obtained in hepatitis and cirrhosis of the liver.^{8,14}

Carotinemia. This is a condition of yellow pigmentation of the blood plasma and selected areas of the skin, due to an increase in carotenoid pigments (carotene).^{16,22} The occurrence of this condition in diabetes (xanthosis diabetica)⁷ and in malnourished children¹⁶ was attributed by many to ingestion of an excessive amount of lipochrome-containing vegetables. That this is not the fundamental cause is shown by the difficulty of production of the condition in healthy subjects by this means.

Carotinemia may occur in diabetes mellitus, advanced chronic glomerulonephritis, myxedema,²³ acute hepatic disease³ and malnutrition with ingestion of unusual quantities of vegetables rich in carotenoid pigments.²⁵ It may be responsible in part for the yellowish or sallow discoloration of the skin in these conditions. It is significant, perhaps, that the clinical disorders in which this condition is prone to develop are also commonly accompanied by lipemia.

It has been suggested that carotinemia (fasting plasma carotene more than 400 micrograms per 100 cc.) occurs when the quantity of carotene reaching the liver exceeds its capacity for storage or conversion of this substance to vitamin A. Some observers have found that the blood carotene curve after

thiamine deficiency and saturation more satisfactorily than does the thiamine content of whole blood.¹⁰ High thiamine values (three times normal in leukocytes, two times normal in erythrocytes) have been obtained in patients with leukemia, Hodgkin's disease and carcinoma of the gastro-intestinal tract.¹ This has been attributed to impaired utilization of thiamine.

After intravenous injection of 50 mg. of thiamine, the level in the serum rises from a control concentration of 0.2 micrograms per 100 cc. to a peak of 130-200 micrograms at five minutes, falling sharply subsequently to 5-15 micrograms at one hour.¹⁶ The rise is greater and more sustained in subjects receiving thiamine, due perhaps to repletion of the body stores of the vitamin.

Thiamine Excretion. Normal limits of excretion of thiamine are difficult to establish because alterations in intake are reflected promptly in its excretion in the urine. Reported values vary also with the method employed, fermentation methods generally yielding higher values than colorimetric procedures and the latter higher results than the rat bradycardia method. The daily urinary excretion by normal subjects receiving adequate amounts of thiamine ranges usually from 60 to 500 micrograms;^{2,19,21a} however, values as low as 25 micrograms have been reported,¹⁰ while some believe that the excretion of less than 90 micrograms is evidence of inadequate intake.¹³ By the fermentation method, values of 240-1327 micrograms have been obtained, 23-75 per cent of which consists, not of thiamine, but of pyrimidines that accelerate yeast fermentation (*PAYF*) and are believed to be products of thiamine degradation.⁹

More satisfactory information regarding the state of thiamine nutrition may be obtained by determining the quantity excreted in the urine after administration of a standard test dose. Several procedures have been suggested.¹⁶ Intravenous injection of 1 mg. of thiamine is followed, in normal adults, by an increment of not less than about 110 micrograms in the urine in the next twenty-four hours, almost all of which appears in the first half-hour. In children under two years more than 250, and from two to five years more than 150 micrograms of the injected thiamine are excreted in twenty-four hours. Very low pre-injection values do not necessarily indicate disease, but may be caused by temporary reduction in thiamine intake. At least 6 per cent of the test dose should be excreted in the urine within four hours after injection of 350 micrograms of thiamine per square meter of body surface.¹⁴ It has been found that after intravenous injection of 1 mg. in normal subjects, free thiamine constitutes more than 60 per cent of the yeast-fermenting substances excreted in

proposed that vitamin B₁ facilitates the synthesis of fat from carbohydrate, presumably through pyruvic acid as an intermediary stage.¹²

There is evidence of the presence of a state of mild vitamin B₁ deficiency in a number of clinical conditions other than beri-beri, including various types of polyneuritis (pregnancy, alcoholism, pernicious anemia, hypochromic anemia, etc.), gastro-intestinal disorders, pregnancy, congestive heart failure, diabetes mellitus and hyperthyroidism.

Several laboratory procedures have been proposed for the quantitative estimation of thiamine and for the detection of thiamine deficiency. These may be classified broadly as biologic and chemical.¹³ The former include: (a) prevention and cure of polyneuritis in pigeons and rats (b) catatorulin effect (oxygen uptake by avitaminous brain tissue from lactate or pyruvate solution); (c) weight maintenance; (d) rat-growth test; (e) rat bradycardia method; (f) growth of *Staphylococcus aureus* or phycomyces. Chemical methods include: (a) the thiochrome method; (b) the reaction with diazotized *p*-amino acetophenone (Prebluda-McCollum reagent) (Melnick and Field);¹⁴ (c) fermentation; (d) determination of bisulfite-binding substances or pyruvic acid. The following determinations have been employed for the clinical detection of thiamine deficiency: (a) the quantity of thiamine in the blood; (b) thiamine excretion in the urine; (c) the quantity of bisulfite-binding substances and of pyruvate in the blood.

Thiamine in Blood. Whole blood contains about 3-10 micrograms of thiamine per 100 cc.,^{9,10} and about 4.5-12 micrograms of cocarboxylase.⁸ Practically all of this is in the cells, the serum in the fasting state containing 0-2 micrograms of thiamine per 100 cc., practically all of which is in the free state.¹⁶ Fermentation methods²⁰ yield somewhat higher values (3-16 micrograms per 100 cc.) than colorimetric methods, due to the presence of other pyrimidines that accelerate yeast fermentation. These may be products of breakdown of thiamine.⁹ The average thiamine content of the leukocytes (20-135 micrograms per 100 cc.) is about ten times as great as that of the erythrocytes (5-15 micrograms per 100 cc.); the same is true of the other pyrimidines that accelerate yeast fermentation.¹⁰ This distribution may be a reflection of the respiratory activity of the leukocytes. Some believe that values below 3 micrograms of thiamine per 100 cc. of whole blood are indicative of deficiency in this factor, subnormal levels having been associated with the development of peripheral neuropathy in alcohol addicts.⁹ It has been found that the thiamine content of the leukocytes reflects states of

quantity of bisulfite-binding substances, is about 0.5-1 mg. per 100 cc. Elevated values are obtained in patients with thiamine deficiency and also in congestive heart failure (to 3.5 mg. per 100 cc.). It has been shown that in normal subjects the ingestion of glucose is followed by a short, steep elevation in blood pyruvic acid, which reaches a maximum in one hour and returns to the resting level in three hours. The curve is abnormally high and prolonged in subjects with thiamine deficiency.⁴⁵ Before a significant increase occurs in the basal pyruvic acid concentration in deficiency states, intravenous injection of 0.4 Gm. of dextrose per kilogram of body weight in 50 per cent solution is followed by an abnormally high rise in blood pyruvic acid (above 1.4 mg. at sixty minutes) and lactic acid (above 1.0 mg. at sixty minutes).²² This phenomenon usually develops some time after "saturation" studies yield abnormal results.

VITAMIN B₂ (RIBOFLAVIN)^{2,4,5,9}

The available evidence suggests that this vitamin, with phosphoric acid and a protein carrier, forms the yellow enzyme of Warburg, which plays an important part in biological oxidation and reduction mechanisms¹ and, therefore, in cell respiration. It has also been suggested that a hormone of the adrenal cortex is essential for the synthesis of this flavin-phosphate compound, and that this, in turn, is necessary for the phosphorylation of hexoses.^{7,12} Failure of such phosphorylation is believed to result in their (hexoses) diminished absorption from the bowel and in abnormalities of their intermediary metabolism.

Riboflavin deficiency in man is evidenced by corneal vascularization, rosacea keratitis, cheilosis, seborrheic lesions about the ears and nose, glossitis and general symptoms.^{3,10,12}

Normal adults on an unrestricted diet excrete 500-800 micrograms of riboflavin daily in the urine.¹¹ As is true of thiamine also, the daily excretion of riboflavin reflects the immediate intake, and subnormal values cannot be regarded as a reliable measure of a state of clinical deficiency in this factor. More satisfactory results in this connection have been obtained by "saturation" tests.^{1,8} When 16 micrograms of riboflavin per kilogram of body weight are injected intravenously, more than 25 per cent (28-68 per cent) of the test dose is excreted in the urine in the next four hours. Excretion of smaller quantities is suggestive of a state of riboflavin deficiency.

NICOTINIC ACID NIACIN)^{5,10}

Nicotinic acid is an essential component of two physiologically important coenzymes, coenzyme I (cozymase) and coen-

the urine during the next twenty-four hours.¹⁸ In a study of unselected hospital patients, 25 per cent excreted less thiamine than other pyrimidines that accelerate yeast fermentation (*PAYF*). It is suggested that low thiamine and normal *PAYF* excretion under these conditions indicates recent thiamine deprivation, while subnormal values for both factors indicate protracted deficiency.

On an intake of 0.4 mg. thiamine per 1000 calories, normal subjects excrete at least 18 per cent of the test dose of 1 mg. (intramuscular injection) in the urine in twenty-four hours.¹⁹ Decreased excretion with this procedure was found to be the earliest indication of thiamine deficiency in two otherwise normal subjects in whom deficiency was induced by restriction of diet.²²

When 0.1 mg. of thiamine per kilogram of body weight is taken by mouth, 8-10 per cent of the test dose is excreted normally in the urine in twenty-four hours, most of it in the first five hours.³

These "saturation" tests have yielded subnormal results in a variety of clinical conditions other than frank thiamine deficiency, including thyrotoxicosis, various neuropathies, diseases of the central nervous system, diarrhea and other gastro-intestinal disorders, congestive heart failure, deficiency in vitamins other than thiamine and chronic diseases of various types. Variable findings have been reported in diabetes. Impaired renal function may result in subnormal excretion in the urine in the absence of thiamine deficiency.

Blood Pyruvic Acid and Bisulfite-binding Substances. The bisulfite-binding power of the blood is an index of the quantity of carbonyl compounds (aldehydes and ketones) including pyruvic acid. These normally range from 2 to 6 mg. per 100 cc., expressed as pyruvic acid. Because the metabolic defect in thiamine deficiency results in the accumulation of pyruvic acid in the organism, this determination was suggested as a diagnostic procedure. However, in addition to frank thiamine deficiency, elevated values have been obtained in patients with congestive heart failure due to organic heart disease, various acute infections, nephrosclerosis and uncontrolled diabetes mellitus. In the last condition, ketone bodies contribute to the increase in bisulfite-binding substances in the blood. Moreover, normal values have been obtained in some cases of thiamine deficiency with elevated pyruvic acid concentrations²⁵ and there is no consistent relation between an increase in these substances and the intensity of symptoms in polyneuritic pigeons.

The normal blood pyruvic acid content, which seems to be more reliable as a measure of thiamine deficiency than is the

is a poor index of vitamin C deficiency, being dependent upon the degree of saturation of the tissues with regard to this factor. This is due to the fact that in the "unsaturated" subject about 99 per cent of the ascorbic acid presented to the kidneys is reabsorbed in the tubules, whereas in the "saturated" subject all the excess of the vitamin ingested above that which can be utilized is excreted.¹⁴

A more satisfactory index of the state of vitamin C nutrition is afforded by the use of the saturation test, involving measurement of the urinary excretion of ascorbic acid following administration of a test dose.^{9,10} The interpretation of this test is based upon the hypothesis that if the vitamin C content of the tissues is subnormal an abnormally large proportion of the administered dose will be retained in the body and, consequently, a smaller amount will be eliminated in the urine than if the vitamin C content of the tissues is normal. A variety of methods have been proposed, including varying amounts of the vitamin, administered orally and intravenously, and a test period of varying duration. The intravenous route of administration and a short test period seem preferable. Following the injection (intravenous) of 100 mg. of ascorbic acid, at least 40 mg. (40 per cent) should be excreted in the urine within three hours in normal subjects. Following the intravenous injection of 1000 mg., at least 400 mg. should be excreted in the urine in five hours. The excretion of quantities less than these is interpreted as indicative of subnormal saturation of the tissues with vitamin C.

There is considerable question as to the significance of results obtained by this procedure. It has been suggested that in some cases abnormal findings may be due to greater utilization of ascorbic acid rather than to subsaturation of the tissues with this factor. Moreover, there is no definite proof of a direct relation between a state of apparent "subsaturation" and the disease conditions in which it may be present, a number of which have been enumerated above. It would appear, however, that the requirement of the organism for vitamin C and the excretion of this factor in the urine can be influenced by a great variety of factors, and that some degree of vitamin C deficiency may contribute to the symptomatology of a number of clinical disorders.

Other Procedures. The ascorbic acid content of the cerebrospinal fluid has been found to range from 0.7 to 2.1 mg. per 100 cc. by some observers and from 1.8 to 4 mg. per cent by others.¹⁷ The concentration in the cerebrospinal fluid appears to vary roughly directly with that in the blood plasma.

determination of the degree of vitamin C saturation of the tissues, consisting in the estimation of the amount of the vitamin excreted in the urine within a stated period following the administration of a test dose.

Vitamin C Content of the Blood. The vitamin C concentration of the blood plasma (reduced ascorbic acid) has been found to range from 0.6 to 2.5 mg. per 100 cc. in normal subjects.⁸ Subnormal concentrations have been found in clinical and experimental scurvy and in a variety of clinical disorders, mentioned above, without frank manifestations of scurvy. Under normal conditions, with an optimum intake of 100 mg. of ascorbic acid, its concentration in the plasma will be maintained at or above 1 mg. per 100 cc. In normal subjects on an adequate diet, the concentration of ascorbic acid in the erythrocytes is about one to two and a half times and in the "white layer" (white blood cells and platelets) twenty to forty times (25-38 mg. per 100 Gm.) that in the plasma.¹ Under normal conditions, the "white layer" contributes approximately as much (0.2 mg. per 100 cc.) ascorbic acid as do the erythrocytes to the whole blood ascorbic acid concentration.

According to some observers, plasma values above 1 mg. per 100 cc. may be regarded as indicating normal vitamin C saturation, and values below 0.6 mg. undersaturation.³ However, values below 0.4 mg. are obtained so frequently in apparently healthy subjects that a diagnosis of subclinical vitamin C deficiency based upon this finding alone is questionable.² The plasma ascorbic acid concentration reacts almost immediately to variations in intake and, therefore, much more reliance is to be placed upon the concentration in whole blood or the white cell-platelet layer.^{1,4,13} In a normal subject given a diet deficient in vitamin C, ascorbic acid fell to zero in the plasma after forty-one days, and in the whole blood and "white layer" after one hundred twenty-four days, the first clinical signs of scurvy appearing on the one hundred thirty-fourth day. Thus, the plasma is the first and the "white layer" the last to be depleted, the latter being apparently the most sensitive index of the state of vitamin C nutrition. It has been suggested that a diagnosis of vitamin C deficiency is warranted when the plasma ascorbic acid concentration is zero and a diagnosis of a prescorbutic state when the whole blood ascorbic acid is zero.¹⁴

Vitamin C in Urine. Normal adults receiving an adequate amount of vitamin C usually excrete 20 mg. or more daily in the urine. In clinical scurvy there is no ascorbic acid in the urine, the traces reported occasionally probably consisting of other reducing substances. The twenty-four-hour excretion of ascorbic acid

isolated from alfalfa and fish meal, respectively. The former has been identified as 2-methyl-3-phytyl-1,4-naphthoquinone. Phthiocol, 2-methyl-3 hydroxy-1,4-naphthoquinone, isolated from the acetone fraction of tubercle bacilli, has been termed vitamin K₃. Vitamin K₄ (synthetic), 2-methyl-1,4-naphthoquinone, is perhaps the most potent of these substances.

So far as is known, the chief function of vitamin K (*Koagulations-vitamin*) lies in its utilization by the liver for the formation of plasma prothrombin. Whereas in certain species, as in the chick; deficiency in this factor can be produced readily by dietary restriction, in most mammals, including man, a substance possessing vitamin K activity is formed by putrefactive bacteria in the intestine and, consequently, clinical manifestations of deficiency (lowered plasma prothrombin) are usually due to either deficient absorption of the vitamin from the intestine or impaired utilization by the liver (p. 425), and, at times perhaps, to increased consumption of prothrombin or vitamin K.

(1) *Impaired Absorption.* Bile acids appear to act as carrying agents for the passage of vitamin K across the intestinal wall, a function similar to that which they perform for vitamin D and carotene. Absorption from the intestine is impaired in the absence of adequate amounts of bile (bile acids) in the intestine, as in obstructive jaundice and external bile fistula, with a consequent fall in plasma prothrombin and development of a hemorrhagic tendency. Adequate absorption under such circumstances follows administration of bile salts, the most effective of which appears to be sodium deoxycholate. Inadequate absorption of vitamin K may occur in conditions of severely disturbed intestinal function, as in patients with intestinal obstruction, intestinal fistula, enterostomy, gastrocolic fistula, chronic pancreatitis, sprue, celiac disease, chronic ulcerative colitis, prolonged diarrhea and anorexia nervosa. Absorption is also interfered with by large amounts of liquid petrolatum.

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Rotter has suggested that instead of determining the concentration of ascorbic acid in the blood plasma or urine by titration with dichlorophenolindophenol, this blue dye may be injected intradermally and the time required for its decolorization noted. He states that when a wheal of 2 mm. diameter is produced by the intradermal injection of a solution of the dye, complete decolorization of the latter should occur in less than five minutes if the tissues are fully saturated with vitamin C. Adequate vitamin C nutrition is indicated by decolorization in from five to ten minutes and vitamin C deficiency is present if more than ten minutes are required. Although other reducing substances, such as glutathione, may cause decolorization of the dye, this procedure may be useful as a rapid clinical test for the demonstration of probable vitamin C deficiency in cases in which the titration method cannot be employed. Its accuracy is, however, questionable.

VITAMIN D

The chief metabolic significance of vitamin D lies in its relation to the metabolism of phosphorus and calcium. The mechanism whereby it produces its characteristic effects in this connection is not completely understood (p. 173), but the rather consistent occurrence of certain characteristic changes renders their demonstration of great practical value in the detection of states of vitamin D deficiency, the most typical of which is rickets. These changes have been discussed in detail elsewhere (pp. 182, 191) and will be only summarized here.

The following findings are obtained characteristically in patients with vitamin D deficiency: (1) diminished serum inorganic phosphorus concentration; (2) a frequently but not invariably diminished serum calcium concentration; (3) increased serum phosphatase activity; (4) decreased corpuscular ester phosphorus; (5) negative calcium and phosphorus balance, with excessive elimination of these elements in the feces.

Hypervitaminosis D is practically never encountered except when deliberately induced experimentally. Its manifestations include hypercalcemia, hyperphosphatemia, diminished serum phosphatase activity and increased excretion of calcium and phosphorus in the urine.

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This is a fat-soluble substance required for the formation of prothrombin, which is an essential factor in the mechanism of coagulation of blood. Many compounds have been found to possess vitamin K activity, two of which, K₁ and K₂, have been

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This failure to respond to this agent has been suggested as a test of liver function (p. 426).

3. *Miscellaneous.* The plasma prothrombin is low in the newborn infant (physiological hypoprothrombinemia) and is one cause of hemorrhagic disease of the newborn. A condition of idiopathic hypoprothrombinemia in adults has been described: Large doses of salicylates and barbiturates may be followed by decrease in plasma prothrombin. Hypoprothrombinemia has been observed in pulmonary tuberculosis, pneumonia, following hemorrhage and after operations, particularly on the biliary tract. In the majority of these conditions, if not accompanied by severe hepatic damage, the prothrombin level increases after administration of adequate doses of vitamin K. Extremely large doses are required to combat the hypoprothrombinemia induced by administration of dicoumarin (3,3'-methylenebis(4-hydroxycoumarin)), a substance responsible for the hypoprothrombinemia that occurs in cattle after eating spoiled sweet clover hay (hemorrhagic sweet clover disease).

Demonstration of Vitamin K Deficiency. The methods employed for the demonstration of vitamin K deficiency consist in determination of the quantity of prothrombin in the plasma. These methods are indirect in nature. The one-stage method (Quick⁵) commonly employed consists in the determination of the clotting time of oxalated plasma at 37.5° C. after addition of an excess of thromboplastin and a fixed amount of calcium. The two-stage method⁶ is more satisfactory but more troublesome. In the first, or prothrombin conversion stage, prothrombin is converted completely to thrombin with an optimal amount of calcium and an excess of thromboplastin. In the second, or clotting stage, the amount of thrombin formed is estimated by the time required for clotting of a standard fibrinogen solution.

Decrease in prothrombin is evidenced by prolongation of the clotting time ("prothrombin time") under these circumstances (see Fig. 15, p. 426). Experience has shown that bleeding occurs commonly in patients with obstructive jaundice, bile fistula, and other conditions mentioned above, when the plasma prothrombin concentration, as measured by these tests, falls below 30-40 per cent of normal. Values of 40-70 per cent of normal are potentially dangerous.

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Chapter XVI

Diabetes Mellitus

RECENT studies have aggravated the long-existing state of dissatisfaction with the view that diabetes mellitus is due solely to failure of the pancreatic islet cells to secrete adequate quantities of insulin. The experimental demonstration of the influence of the pituitary and adrenal glands, the liver and other extra-pancreatic tissues upon carbohydrate metabolism has led to attempts to involve these organs in the etiology and pathogenesis of clinical diabetes mellitus. Among the hypotheses that have been advanced may be mentioned the following: (1) that diabetes is due in some instances to excessive secretion of the "diabetogenic hormone" of the anterior hypophysis; (2) that it may be dependent upon imperfect inhibition of excessive glycogenolysis and excessive gluconeogenesis; (3) that it may be due in some instances to deficient formation of an hypothetical insulin-activator or insulin kinase, secreted perhaps by the liver or duodenum. Although there is no direct evidence of the validity of such hypotheses, the data at hand are highly suggestive of the participation of some extra-insular factor in the etiology of clinical diabetes mellitus, and the view is constantly becoming more definitely justified that this condition may have its fundamental origin in something other than a mere state of hypoinsulinism. However, despite the lack of exact knowledge regarding the pathogenesis of clinical diabetes mellitus, considerable information is available regarding the pathologic physiology of this condition. Perhaps in no other clinical disorder are diagnosis and proper treatment so completely dependent upon careful and continued investigation of certain metabolic manifestations of the disease process.

DECREASED RESPIRATORY QUOTIENT

The respiratory quotient is an index of the relative percentages of fat, protein and carbohydrate oxidized in the organism during the experimental period (see p. 302). The R.Q. for carbohydrate being 1.0, that for protein being 0.8 and for fat 0.7, it is generally assumed that a decrease in the respiratory quotient from the value characteristic of normal subjects on a mixed diet under resting conditions, 0.85, approaching 0.7 in

complete diabetes, is indicative of the degree of impairment of carbohydrate utilization in this condition. Muscular exercise, epinephrine and glucose, which cause a rise in the respiratory quotient of normal individuals, usually have no such effect in untreated diabetic patients. In some instances there may actually be a diminution in the respiratory quotient following the ingestion of glucose. Certain factors, such as the general state of nutrition and the degree of acidosis, exert an influence upon the respiratory quotient values, which, however, constitute perhaps the most satisfactory index of the severity of the condition, improvement being indicated by a rise in the respiratory quotient. It should be mentioned here that some authorities believe that overproduction of glucose (from protein and fat) rather than impaired utilization of glucose constitutes the fundamental abnormality of carbohydrate metabolism in diabetes mellitus.¹² The R.Q. for the conversion of protein to glucose has been estimated as 0.632 to 0.706, that for gluconeogenesis from fat being about 0.281. According to the proponents of the overproduction theory, the usual diabetic R.Q., approaching 0.7, is regarded as the resultant of two processes proceeding simultaneously, (a) gluconeogenesis from protein and fat, with an R.Q. ranging from 0.2 to 0.7 and (b) the oxidation of carbohydrate, with an R.Q. of 1.0.

HYPERGLYCEMIA

Fasting hyperglycemia is one of the most important diagnostic features of diabetes mellitus. It occurs as a result of the fact that the rate of withdrawal of glucose from the blood is diminished because of the impaired ability of the tissues to store glycogen. The degree of hyperglycemia is not, however, an accurate index of the severity of the condition because of the influence of such added factors as age, the state of nutrition, the available carbohydrate supply, the degree of acidosis, the presence of infection and the state of hepatic and renal function. In mild cases the fasting blood sugar may be within normal limits (65-110 mg. per 100 cc.), the existing disturbance of carbohydrate metabolism being evidenced only by the application of such procedures as the determination of the tolerance to glucose or the respiratory quotient. In moderately severe cases values as high as 300 mg. per 100 cc. may be obtained. In severe cases the fasting blood sugar level may rise to 600 mg. per 100 cc. or higher, values as high as 2000 mg. having been reported.

There is a remarkably wide range of variability in blood sugar values in patients in diabetic coma. This condition has been present with blood sugar concentrations as low as 130 mg. per

100 cc. and has been absent with values as high as 1500 mg. per 100 cc. In an analysis of a large series of cases, Baker found that coma is most likely to develop in the presence of relatively low blood sugar values in patients below the age of twelve years and above the age of fifty years, and in those with acute and overwhelming infection. Moreover, there appears to be no consistent relationship between the blood sugar concentration and the occurrence of death or recovery in patients with diabetic coma. Death may occur with values below 300 mg. per 100 cc. and recovery has been reported in patients with blood sugar concentrations as high as 1800 mg. per 100 cc.^{3,5} Obviously, the blood sugar concentration alone is not a reliable criterion for judging the severity of diabetic coma in any individual case.

DECREASED CARBOHYDRATE TOLERANCE (See p. 38)

Diminution in the tolerance of the organism to glucose ranks in importance with decrease in the respiratory quotient in the diagnosis of diabetes mellitus. It is of particular value, clinically, in those cases in which the fasting blood sugar is within normal limits. A diagnosis of diabetes mellitus cannot be made in the absence of a decrease in the carbohydrate tolerance of the organism. The abnormally high and prolonged blood sugar curve following the ingestion of glucose (p. 38) is characteristically associated with a diminished arterial-venous blood sugar difference and with a diminution in the degree to which the blood inorganic phosphate falls during the period of increased carbohydrate supply. These phenomena are generally interpreted as indicating diminished tissue utilization of glucose. The tolerance to levulose is likewise diminished but not to the same extent as that for glucose. The glucose tolerance test is useful only in mild or moderately severe cases of diabetes mellitus where the diagnosis may be in doubt, but it should not be applied in severe cases in which the administration of large quantities of glucose may be not without danger.

As stated elsewhere (p. 37), the belief that abnormalities in the glucose tolerance curve in patients with diabetes mellitus are indicative of a diminished quantity or activity of insulin, or that they can be interpreted in terms of diminished utilization of glucose has been subjected to considerable opposition in recent years.^{4,13} Some believe that the excessively high and prolonged tolerance curve is more directly dependent upon excessive glycogenolysis in the liver than upon impaired glucose utilization in the tissues. Moreover, observations upon depancreatized animals that have been also hypophysectomized or adrenalectomized indicate clearly that the organism is capable of

utilizing glucose in the absence of insulin. However, whether one subscribes to the overproduction or underutilization theory of the pathogenesis of diabetes mellitus, clinical experience has demonstrated the practical diagnostic importance of the glucose tolerance test. Joslin states that it is his rule to regard a blood sugar concentration of 170 mg. per 100 cc. or more following a meal as indicative of diabetes mellitus. If capillary blood is used for the sugar determination the critical diagnostical level is raised to 200 mg. per 100 cc.

GLYCOSURIA (See p. 65)

Glucose is eliminated in the urine when the blood sugar level rises above the renal threshold for glucose which, under normal conditions, ranges from 160 to 180 mg. per 100 cc. Since in mild cases of diabetes the fasting blood sugar level may be below this point, glycosuria may occur intermittently, chiefly following the ingestion of carbohydrate-rich meals. Under such circumstances it is obvious that single specimens of urine may not contain glucose and it is important that the examination be conducted on twenty-four-hour specimens of urine or on all voided specimens. In more severe cases, the blood sugar being maintained at a relatively high level, glycosuria of varying degree occurs continuously. In mild cases the urine (twenty-four-hour sample) may contain from a trace to 1 per cent of glucose; moderately severe cases, 1 to 3 per cent; severe cases, 3 to 8 per cent. Concentrations higher than 8 per cent are rarely observed because of the difficulty of maintaining larger amounts of glucose in solution in the urine. The kidney responds to the necessity for the elimination of larger quantities of glucose by the simultaneous excretion of increased quantities of water. Polyuria is almost invariably observed as the concentration of glucose in the urine rises above 4 per cent. Under such circumstances, if water is supplied in abundance, extremely large volumes of urine may be passed daily, occasionally as much as 2 or 3 gallons. The elimination of such large quantities of water frequently results in extreme grades of dehydration. One of the characteristic features of the polyuria of diabetes mellitus is the high specific gravity of the urine, frequently 1.030 or more, which is due to its high sugar content. This feature distinguishes it from polyuria due to other conditions in which the specific gravity of the urine is usually in inverse proportion to the urine volume.

In some cases glycosuria may be absent although the blood sugar concentration is above the normal "renal threshold" level (see p. 55) and, at times, may be extremely high. The occurrence of hyperglycemia without glycosuria is ascribed to an

increase in the "renal threshold" for sugar and is observed most commonly in association with arteriosclerosis or with nephritis in elderly individuals with diabetes of long standing. Sudden, marked variations in the "renal threshold" for sugar may occur in such individuals, a phenomenon for which no entirely satisfactory explanation has been established. (p. 58).

LIPEMIA (See p. 149)

As the impairment of carbohydrate utilization progresses in diabetes, the metabolism of fat is proportionately increased in order to maintain the heat requirements of the organism. This increase in the intermediary metabolism of fat is accompanied by an increase in the concentration of lipids in the blood. The blood plasma normally contains 140 to 250 mg. of cholesterol per 100 cc., 190 to 450 mg. of fatty acids, 0 to 370 mg. of neutral fat and 60 to 350 mg. of phospholipid. Values for total blood fat as high as 25 per cent have been observed; figures above 10 per cent are extremely unusual. From a clinical standpoint the degree of hypercholesterolemia may be accepted as usually indicative of the degree of increase in the total lipid concentration in diabetes. Although there is no definite parallelism between cholesterolemia and glycemia, glycosuria, ketonuria or acidosis, many believe that, particularly in untreated cases, the degree of cholesterolemia is a more satisfactory index of the severity of the diabetic condition than is any one of the other factors. Plasma cholesterol values above 1000 mg. per 100 cc. are occasionally observed, the usual range being from 250 to 400 mg. per 100 cc. In many cases the plasma cholesterol remains above normal for varying periods of time after the blood sugar has fallen to within normal limits. It therefore serves as a valuable measure of the efficacy of therapeutic procedures and as an indication of the necessity for further treatment.

The various opinions regarding the cause and significance of hypercholesterolemia in diabetes mellitus have been considered elsewhere (p. 149). It is believed by some that hemoconcentration plays an important part in the production of this phenomenon. It must be kept in mind that hypocholesterolemia, with or without increase in other blood lipids, may occur in severe forms of the disease, particularly in terminal states. The cause of this phenomenon is not known (p. 161).

KETOSIS (See p. 161)

According to the prevailing concept, as carbohydrate utilization diminishes and the combustion of fat correspondingly increases, a point is eventually reached at which the oxidation

of fatty acids becomes impaired. It has been found that the oxidation of 1 Gm. of glucose is required for the simultaneous complete oxidation of 1.5 Gm. of fatty acid, the so-called "ketogenic-antiketogenic" (fatty acid-glucose) ratio being 1.5. If this ratio exceeds this figure, fatty acids cannot be completely oxidized, the process stopping at the 4 carbon atom stage, with the production of aceto-acetic acid, from which are formed betahydroxybutyric acid and acetone. As the saying goes, fat burns in the fire of carbohydrates and in the absence of the latter it smokes, the smoke being represented by the ketone bodies. The ketone content of blood is seldom determined, the appearance of these bodies in the urine being a satisfactory though not exact indication of the existence of a state of ketosis in diabetes. Because of the intimate relationship between ketosis and acidosis in diabetes mellitus, the occurrence of ketonuria should constitute a danger signal and an indication of the necessity for prompt and active therapy.

As has been indicated elsewhere (p. 336), considerable objection has been raised to the view that ketone formation and accumulation in diabetes mellitus are dependent directly upon impaired combustion of these substances in the tissues. There is strong experimental evidence that ketone bodies are formed only in the liver; consequently, the antiketogenic effect of insulin and glucose cannot be related to the utilization of carbohydrate in the tissues generally, but must be due to an effect produced by these agents in the liver. Mirsky believes that a diminution in liver glycogen is probably the essential stimulus to ketone formation by the liver and that any agent that inhibits excessive breakdown of liver glycogen also prevents the formation of ketone bodies. The concept advanced by this school of thought is as follows: The ketone bodies are in themselves end-products of fat metabolism in the liver. This organ preferentially burns carbohydrate, but in the absence of adequate quantities of carbohydrate, fat and protein are oxidized in excess. When this occurs, ketone bodies, as end-products of fat metabolism in the liver, pass into the blood stream and are utilized by the extra-hepatic tissues. The degree of resulting ketonemia and ketonuria depends upon the difference between the rate of ketone formation and the rate of ketone utilization. According to this concept, instead of facilitating fat metabolism by its combustion, glucose actually inhibits it. The essential cause of ketosis in diabetes mellitus would therefore be depletion of liver glycogen as a result of excessive hepatic glycogenolysis and an inability on the part of the liver to store adequate quantities of this substance (Fig. 3, p. 163).

ACIDOSIS (See p. 279)

Acidosis in diabetes is due in part to the existing ketosis and in part to the excessive loss of water with the associated elimination of excessively large quantities of base. Diabetic acidosis, therefore, is due to a primary alkali deficit which is most satisfactorily detected by one of the procedures designed to measure the alkali reserve of the body. Perhaps the most valuable of these from a clinical standpoint is the determination of the CO_2 combining power of the blood plasma which, in diabetes, may be reduced to a remarkable degree, the extent of reduction being more or less accurately indicative of the degree of acidosis. As a rule, coma is almost always present when the CO_2 combining power is less than 15 volumes per cent, but the individual threshold of consciousness varies within rather wide limits. Patients have been observed in profound diabetic coma with values of 28 volumes per cent while others with values of 10 to 12 volumes per cent have been merely drowsy or slightly stuporous. Although the plasma CO_2 capacity more nearly parallels the clinical condition of patients with severe diabetes than does the blood sugar concentration, values below a certain critical level appear to have little significance with regard to prognosis. According to Baker, this critical level is about 25 volumes per cent. He believes that the presence or absence of infection complicating diabetic coma seems to be of greater consideration than do values for blood sugar or the alkali reserve in determining the prognosis in any given instance. Since the introduction of insulin therapy, recovery from diabetic coma has been reported in patients with values for plasma CO_2 combining power as low as 2 volumes per cent.

HYPOCHLOREMIA AND HEMOCONCENTRATION

Although the plasma chloride concentration is usually within normal limits in diabetes mellitus, hypochloremia is occasionally observed, particularly in advanced forms of the disease. It is now recognized that severe diabetes is accompanied by profound changes in electrolyte balance and in the electrolyte pattern of the blood. It has been found that the polyuria which attends the increasing glycosuria is accompanied by an equally pronounced increase in the excretion of electrolytes, particularly sodium and chloride. It appears that the initial loss of base under such circumstances is not dependent primarily upon the development of ketosis, but rather accompanies the appearance of marked glycosuria and polyuria.¹ With the progressive development of ketosis and acidosis, there is a secondary increase in the loss of

water and electrolytes which continues until the condition is brought under control by proper therapeutic measures.⁷

The following concurrent phenomena are believed to constitute the mechanism underlying the production of changes in the electrolyte pattern of the blood in diabetic acidosis:¹¹ (1) Displacement of CO_2 from bicarbonate by ketone acids. (2) Although a portion of the ketone acids can be eliminated as free acids and a portion is neutralized by ammonia, a certain fraction is neutralized by fixed base. The excretion of the latter fraction, in contradistinction to the former, withdraws base from the body which can be replaced only from extraneous sources. (3) Reduction of base is equivalent to reduction of the total electrolyte content of the body fluids and, consequently, dehydration, resulting from the simultaneous loss of body fluid. (4) Chloride depletion is apparently related more directly to glycosuria and polyuria than to acidosis.

It should be noted that in the presence of dehydration the actual deficiency in body chloride is much greater than would appear to be indicated by the plasma chloride concentration and far exceeds the deficiency in bicarbonate. In fact, it has been shown that because of the concomitant dehydration and hemoconcentration it is quite possible for the plasma chloride level to be actually elevated following a period of actual negative chloride balance and in the presence of marked chloride depletion. These observations have important therapeutic implications.⁷ In the presence of acidosis, as stated elsewhere (p. 237), passage of chloride from the plasma to the red blood cells may contribute to the development of a decrease in the plasma chloride concentration.

HYPOPROTEINEMIA

Patients with diabetes mellitus are still encountered occasionally in whom injudicious dietary restriction has resulted in a state of extreme malnutrition, with diminution in the plasma protein concentration and, at times, edema. During periods of severe acidosis, particularly if accompanied by vomiting, the extent of reduction of the plasma protein concentration may be masked to a large extent by the dehydrating effects of vomiting and acidosis, with resulting hemoconcentration. If a patient in this condition were treated actively by the administration of adequate quantities of glucose, insulin, fluid and sodium chloride, the restoration of normal plasma volume would be accompanied by a more or less marked diminution in the concentration of plasma protein, which may be so low that edema develops. The development of this phenomenon is not uncommon following

active treatment of patients in diabetic coma, with severe acidosis. The occurrence of albuminuria, hypoproteinemia, impairment of renal function and hypertension in patients with diabetes mellitus has been found to be associated with morphologic changes in the kidneys of a peculiar type, designated "intercapillary glomerulosclerosis."

NITROGEN RETENTION

A moderate to marked increase in the nonprotein nitrogenous constituents of the blood may occur in patients with advanced diabetes mellitus, particularly in those in diabetic coma.⁸ In some instances this is due to the presence of complicating renal disease, such as intercapillary glomerulosclerosis, glomerulonephritis, advanced nephrosclerosis or marked degenerative changes in the kidneys. In other instances, however, no significant renal lesions have been demonstrable at autopsy. The cause of nitrogen retention under such circumstances is not known, but it may be dependent upon the operation of one or more of several factors. Among these are: (1) dehydration, due to acidosis, diuresis, vomiting and limited fluid intake; (2) circulatory failure and extremely low arterial blood pressure (state of shock); (3) excessive catabolism of protein; (4) hypochloremia, with low plasma sodium concentration. No one of these factors has been shown to be operative in all cases manifesting this phenomenon.

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Chapter XVII

Renal Function

MODERN physiologic investigations have contributed much to our understanding of the functions of the kidney and, to a lesser extent, to our knowledge of the mechanisms involved in the performance of these functions. Since the original observations of Bowman, in 1842, opinion regarding the essential nature of renal functional activity has been divided, some adhering to the "mechanistic" theory of Ludwig (1844) and others to the "vital" (secretory) theory of Heidenhain (1874). With advancement in our understanding of physical chemical processes it has become increasingly apparent that, in the words of Claude Bernard, "There is in reality only one general physics, only one chemistry, and only one mechanics, in which all the phenomenal manifestations of nature are included, both those of living bodies as well as those of inanimate ones. In a word, all the phenomena which make their appearance in a living being obey the same laws as those outside of it." The existing gaps in our physiologic knowledge are due to the fact that, as stated by Bayliss, no physicochemical system has been encountered, up to the present time, which has the same properties as those known as vital; in other words, "none have, as yet, been prepared of similar complexity and internal co-ordination."

The chief function of the kidneys is to remove from the body waste and other undesirable substances and whatever water and solid material may have been formed in or introduced into the body in excess of the quantity required. They constitute an important route of elimination of certain drugs, poisons and other toxic agents. Formation of ammonia by the renal tubular epithelium and regulation of the excretions of anions and cations play an important part in the regulation of the acid-base equilibrium (p. 275). By virtue of these functions, the kidneys also play an essential role in the regulation of water balance and the osmotic equilibrium between the blood plasma and the tissue fluids and cells. The uriniferous tubule is the functional unit, there being about 1,200,000 in each kidney. From a physiologic standpoint, the activity of the glomerulus must be considered apart from that of the tubule.

GLOMERULAR FILTRATION

The work of Richards and his associates^{42, 46} substantiates the hypothesis of glomerular filtration proposed by Ludwig and demonstrates conclusively that glomerular urine formation is dependent upon circulatory conditions in the glomerular capillaries (pressure and rate and volume of blood flow). These investigators have demonstrated another fact of great significance; namely, that "the number of glomeruli through which the blood flows and hence which function at any one time may be a fraction only of the total number of glomeruli in the kidney" and that, therefore, "the extent of filtration surface in the kidney is variable and a factor which must be of major importance in the adjustment of renal function to excretory requirement."

Studies of glomerular fluid and serum filtrate (protein-free) in amphibians have shown that the pH, vapor pressure, conductivity, and concentrations of urea, glucose, chloride, inorganic phosphorus, exogenous uric acid, exogenous creatinine, bicarbonate, etc. in these fluids are practically identical within the error of the methods employed. These observations indicate that, at least in certain amphibians, the glomerulus acts merely as an ultrafilter. There seems to be little doubt of the applicability of these facts to problems of renal functional activity in mammals. According to this view, therefore, which rests upon sound experimental evidence, glomerular urine is formed by a process of filtration alone. The effective filtration pressure is the resultant of the blood pressure in the glomerular capillaries and the opposing forces of the colloid osmotic pressure of the blood plasma and the tension within Bowman's capsule (capsular pressure). The mean glomerular pressure may be regarded as about 50 per cent of the mean systemic arterial pressure (90 mm. Hg), thus averaging about 45 mm. Hg. This is subject to regulation by variation in the relative degree of constriction of the afferent and efferent arterioles of the glomerulus. Under stimulation, the latter may be constricted more than the former to such a degree as to raise the pressure within the glomerular capillaries to about 63 mm. Hg. If it is assumed that the colloid osmotic pressure is 24 mm. Hg in the blood entering the glomerulus, and that the capsular pressure is about 15 mm. Hg, it is obvious that the initial effective filtration pressure is at least 45 minus (24 + 15) or 6 mm. Hg. and may rise to 63 minus (24 + 15) or 24 mm. Hg. As fluid (and diffusible solids, but little or no protein) leaves the plasma within the capillaries as a result of this force, the plasma protein concen-

tration increases and may reach a point where the effective filtration pressure is so reduced as to impair glomerular filtration.

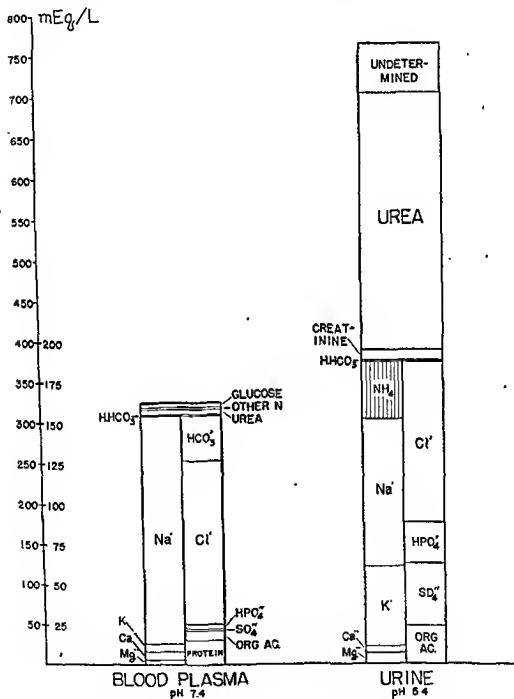


Fig. 13.—Illustrating the manner in which the kidney defends the chemical pattern of the blood plasma, producing a substance (urine) differing widely and variably from blood plasma as regards the relative quantities of substances, osmotic value and reaction. The figures to the left (ordinate) indicate the sum of the milliequivalents of acid and basic ions (*Gamble.*)¹¹⁰

Further constriction of the efferent arteriole may then still increase the glomerular blood pressure (to 63 mm. Hg), allowing filtration to continue. In addition to the influence of these

pressure factors, the volume of glomerular filtrate is influenced significantly by (a) the surface area of the filter (glomerular capillary surface, normally 1.56 sq. meters) and (b) the minute volume flow of blood plasma over this surface (normally about 700 cc. plasma or 1200 cc. blood per 1.73 sq. meters body surface). These factors are affected by such conditions as glomerulonephritis, extensive destructive lesions of the kidneys, advanced nephrosclerosis and, at times, congestive heart failure. Under normal conditions, the volume of glomerular filtrate averages about 125 cc. per minute. This will be discussed in detail in connection with the discussion of inulin tolerance (p. 350).

It is apparent that glomerular filtration may be diminished, with a tendency toward retention of waste products in the blood, in the absence of primary morphologic changes in the kidneys, by (1) extrarenal factors which diminish the renal blood flow and (2) factors which lower the effective filtration pressure by (a) decreasing the glomerular blood pressure, (b) increasing the plasma oncotic pressure (p. 77) or (c) increasing the capsular pressure. Among the most important of these are clinical conditions characterized by marked lowering of the systemic blood pressure, dehydration or hemoconcentration and cardiac failure (coronary artery occlusion, duodenal and pyloric obstruction, peritonitis, severe hemorrhage, Addison's disease, pneumonia, severe diarrhea, acute pancreatitis, perforated peptic ulcer or gallbladder, diabetic coma, extensive burns, left ventricular failure, urinary tract obstruction and various clinical states of shock. In such conditions, as in any condition of renal functional impairment, the tendency toward nitrogen retention may be aggravated by a simultaneous increase in protein catabolism or absorption of excessive amounts of protein end-products into the organism (e.g., in massive gastro-intestinal hemorrhage).⁹

TUBULAR FUNCTION

If, as has been estimated, the rate of formation of glomerular urine under normal conditions is approximately 125 cc. per minute, while the rate at which urine passes into the bladder under the same conditions is approximately 1-2 cc. per minute, it is obvious that in its passage through the uriniferous tubules about 99 per cent of the water of the glomerular filtrate must have been reabsorbed. Furthermore, since the glomerular filtrate contains glucose in practically the same concentration as the blood plasma, whereas the bladder urine contains none, or very little, this substance, too, must have undergone practically complete reabsorption in the tubules. Quantitative studies of

the excretion of other solids, such as chloride, phosphate, and bicarbonate, in glomerular and bladder urine indicate a variable degree of reabsorption during their passage through the uriniferous tubules. The important observations of Richards and Walker and their associates have thrown considerable light upon the site of reabsorption of several of these solids in the renal tubules of amphibians. It was found that reabsorption of glucose occurs entirely in the proximal convoluted tubule, (the degree of reabsorption of glucose was lessened by an increase in the rate of urine flow through the tubule and by high concentrations of glucose in the blood plasma) reabsorption of chloride in the distal tubule, and acidification of the urine in the terminal portion of the distal tubule, probably as a result of reabsorption of bicarbonate. The proximal tubule appeared to be capable of actively reabsorbing phosphate.

Insofar as excretory functions are concerned, therefore, the function of the renal glomeruli may be regarded as that of ultra-filtration (protein-free filtrate of the blood plasma) and that of the tubular epithelium as chiefly "selective" reabsorption (water, glucose, chloride, etc.).^{42,55} However, there is evidence that certain of the urinary constituents pass from the blood into the urine at some point in the tubules beyond the glomeruli. There appears to be a distinct species difference in this regard, but it is probable that creatinine, ammonia, diodrast, phenol red and hippuran are added to the urine in the course of its passage through the tubules. Inasmuch as the average rate of glomerular filtration is 120-130 cc. per minute (about 70 cc. per sq. meter of body surface) (p. 350), it follows that more than 170 liters are filtered through the glomeruli from the plasma daily, the tubules subsequently reabsorbing about 168.5 liters of water, 1000 Gm. NaCl, 360 Gm. NaHCO_3 , 170 Gm. glucose and smaller amounts of phosphate, sulfate, amino acids, urea, urate, etc., in order to excrete about 60 Gm. of NaCl, urea and other waste products in about 1500 cc. of urine.⁴⁴

The factors involved in the regulation of tubular reabsorption are not clearly understood, but there is evidence that the total quantity of any substance present in the glomerular filtrate is important in this connection. For example, under normal conditions the tubules can reabsorb up to 350 mg. of glucose per minute (p. 57); this would be the quantity entering the tubules with a glomerular filtration rate of 125 cc. per minute and a plasma glucose concentration of 280 mg. per 100 cc. The same quantity would enter the tubules per minute if the volume of glomerular filtrate was reduced to 75 cc. per minute and the plasma glucose concentration raised to 465 mg. per 100 cc. If

tubular reabsorption was not impaired simultaneously, glucose would not necessarily escape in the urine regardless of the considerable elevation of blood sugar. The extent to which the various constituents of the glomerular filtrate are reabsorbed may be ascertained by determining their "clearance" simultaneously with the clearance of inulin (p. 350). By this method it has been found that 40-50 per cent of the urea and about 90 per cent of the urate (uric acid) of the glomerular filtrate is reabsorbed in the tubules under normal conditions. In the course of the reabsorption of the several solid constituents, about 80 per cent of the water entering the proximal convoluted tubules and loop of Henle is reabsorbed isosmotically, a phenomenon designated "obligatory reabsorption."^{12,43,44} A variable proportion of the remaining 20 per cent is reabsorbed ("facultative reabsorption")^{12,43,44} probably as a result of the action of the "antidiuretic hormone" of the pituitary gland, which exerts its influence directly upon the renal tubular epithelium. The first (obligatory) phase is constant and is determined by the extent of reabsorption of solids; the second (facultative) phase is variable, being subject to alteration in physiologic demands, and constitutes the mechanism of production of a urine which is hypertonic as compared to protein-free blood plasma. Hyposthenuria (decrease in maximum attainable specific gravity) results from decreased efficiency of facultative reabsorption, which, when extreme, results in isosthenuria (p. 384).

The kidneys have at least one important synthesizing function, namely that of ammonia formation. As indicated elsewhere (p. 84), ammonia is formed, apparently from amino acids, through the activity of the renal tubular epithelium, chiefly in response to and in proportion to the requirement for conservation of base. The substitution of ammonia for sodium and potassium in the excretion of acid radicles in the urine effects an economy of base which is essential for the maintenance of the normal pH of the body fluids under conditions of excessive accumulation of acids in the body. The exact mechanism of formation of ammonia from amino acids in the kidney and the exact stimulus to its increased production under such circumstances are not clearly understood. However, this function of the kidney plays an important part in the mechanism of regulation of the acid-base balance of the organism.

Although not strictly accurate from a physiologic standpoint, from a practical standpoint the chief functions of the kidney which have a significant clinical bearing may be enumerated as follows:

(1) The elimination of water in accordance with the requirements of the organism.

(2) The elimination of salts (chloride, phosphate, etc.) in accordance with the requirements of the organism.

(3) The elimination of nonvolatile end-products of metabolism, chiefly those of protein metabolism.

(4) The elimination of certain foreign substances (foreign proteins, dyes, etc.)

(5) The synthesis of hippuric acid and ammonia formation.

(6) The retention of normal protein constituents in the blood plasma and the reabsorption, in the tubules, of substances necessary to the organism which pass in excessive quantities into the glomerular filtrate (glucose, chloride, etc.).

As a result of these functions the kidneys play an important part in:

(1) The regulation of the water balance and the crystalloid and colloid osmotic equilibrium between the blood plasma and tissues.

(2) The regulation of the acid-base equilibrium of the body.

(3) The removal of toxic substances and waste products.

Considered in a broad sense, the chief function of the kidney is to eliminate solid substances in solution in water. Many of these substances exist in the urine in much greater concentration than in the blood, the ratio of the average concentration in the urine to its concentration in the blood during the same period (concentration ratio) varying greatly for each of the urinary constituents. This is illustrated in the following table (Fishberg). Thus, in the normal performance of its excretory functions the

TABLE 8
RELATIVE CONCENTRATIONS OF CONSTITUENTS OF URINE AND BLOOD

Substance	Concentration in urine, mg. per 100 cc.	Concentration in blood, mg per 100 cc	Concentration ratio	Concentration in blood in renal insufficiency
Urea ..	2000	30	65	Increased
Uric acid	60	2	30	Increased
Creatinine	75	2	35	Increased
Indican.	1	0.05	20	Increased
Phosphate	150	3	50	Increased
Sulfate..	150	3	50	Increased
Potassium	150	20	7	Increased slightly
Chloride	500	350	1.5	Not increased
Sodium	350	335	1	Not increased
Calcium	15	10	1.5	Not increased
Water.				

kidney must concentrate the eliminated substances, the necessary degree of concentration at any moment depending upon the relative quantities of solids and water available at that moment in the blood passing through the glomerular capillaries. One of the most important characteristics of the healthy kidney is its ability to eliminate the required quantity of solids regardless, within wide limits, of the amount of water available for their solution. In other words, the normal kidney exhibits a remarkable flexibility in its concentrating ability. Consequently the concentration of solid constituents of normal urine, as evidenced by the specific gravity, varies considerably during the day in accordance with the ingestion of fluids and solid food and with the metabolic activity of the tissues. If large amounts of fluids are ingested the urine is of large volume and low specific gravity; if little water is ingested, or large amounts are lost through other channels such as the skin and gastro-intestinal tract, the urine is of small volume and high specific gravity. The investigation of the concentrating ability of the kidney constitutes a most valuable measure for the determination of renal functional integrity.

DETERMINATION OF THE INTEGRITY OF THE INDIVIDUAL FUNCTIONS OF THE KIDNEY

The study of renal function may be approached from the standpoint of the individual functions of the kidney. This method of investigation was formerly considered by many to be essential for the complete understanding of the state of renal activity, because of the prevalent belief in the existence of selective injury to individual functions of the kidney in various types of nephritis. Two main types of chronic nephritis were recognized: that with nitrogen retention, also termed azotemic, uremigenic and hypoazoturic and that with salt and water retention, also termed hydropigenic and hypochloruric. This distinction can no longer be considered tenable, and the hypothesis of selective impairment of individual functions of the kidney has been largely superseded by the conception of the unitary nature of impairment of renal function. Modern studies have clearly demonstrated that variations in the clinical picture of renal insufficiency are dependent largely not upon renal but upon extrarenal factors such as variations in the acid-base balance and in the crystalloid and colloid osmotic balance of the blood and tissues. The study of individual functions of the kidney should not, therefore, be undertaken with the view of differentiating thereby several types of renal injury. This method of investigation is, however, of importance in many instances

from the standpoints of diagnosis, prognosis and treatment, and will therefore be considered in detail.

CLEARANCE TESTS

In the performance of its excretory functions, the kidney may be said to "clear" the blood of certain waste and foreign products. Möller, McIntosh and Van Slyke³⁵ defined urea clearance as the volume of blood which one minute's excretion of urine suffices to clear of urea. Inasmuch as all of the blood flowing through the kidneys is only partially cleared of urea (and other substances), a more exact definition would be "the number of cubic centimeters of blood which contain the amount of urea removed per minute by renal excretion." As pointed out by Smith,⁴⁴ the clearance also represents the minimum volume of blood required to furnish the amount of substance excreted in the urine in one minute. This concept of renal clearance has been expanded to include a number of substances other than urea and has contributed largely to our present understanding of renal function in health and disease.

If U indicates the concentration (mg. per 100 cc.) of substance (e.g., urea) in urine, and V the volume (in cc.) of urine formed per minute, $U \times V$ equals the quantity of substance excreted per minute. If B indicates the concentration (mg. per 100 cc.) of substance in the blood, UV/B indicates the virtual volume of blood "cleared" of substance per minute, i.e., the "clearance." It is essential that plasma be used instead of whole blood for clearance determinations if the substance investigated is not distributed uniformly between plasma and corpuscles (e.g., inulin, diodrast, phenol red).

Theoretically, a substance may be excreted by (a) glomerular filtration alone, (b) filtration plus tubular excretion or (c) filtration plus tubular reabsorption.⁴⁴ If a substance is completely filtered at the glomerulus and is subsequently completely reabsorbed by the tubules, its clearance will be zero (e.g., glucose). As the degree of tubular reabsorption diminishes, the substance appears in the urine and its clearance increases (e.g., urea), until, if there is no reabsorption (e.g., inulin), the clearance will be equivalent to the rate of glomerular filtration. If a substance, in addition to being filtered through the glomeruli, is also excreted by the tubular epithelium (e.g., phenol red, diodrast), its clearance will exceed the rate of glomerular filtration by an amount equal to the extent of tubular clearance. Inasmuch as the kidneys cannot excrete more of a substance per unit of time than is brought to them in the blood, the upper limit of renal clearance is determined by the renal blood flow.

For example, if a substance undergoes glomerular filtration and tubular excretion, and if all that is contained in the blood passing through the kidneys is removed and is concurrently transferred to the urine, its clearance will be complete (e.g., diodrast clearance); i.e., it will be equivalent to the volume of plasma flowing through the kidneys per minute.⁴⁴ These facts constitute the basis for quantitative determination of various aspects of renal function.

Glomerular Filtration—Inulin Clearance. Inulin is a polysaccharide which is not metabolized in the body and, following its intravenous injection, is excreted quantitatively by the kidneys within a short time. It is excreted entirely by glomerular filtration and undergoes no reabsorption in the tubules. Inasmuch as inulin, being freely filtrable at the glomerulus, exists in the blood plasma and glomerular filtrate in identical concentration, and since the quantity of inulin excreted per minute in the bladder urine is equal to the amount entering the glomerular filtrate per minute, it follows that the inulin clearance (UV/P) represents the volume of glomerular filtrate formed per minute. Employing this procedure, it has been found that the average rate of glomerular filtration is 125 cc. per minute (about 70 cc. per sq. meter of body surface).

Determination of the rate of glomerular filtration (inulin clearance) has several important physiologic applications:⁴⁵ (a) the glomerular filtration rate minus the rate of urine flow (bladder) equals the quantity of water reabsorbed in the tubules per minute; (b) the inulin clearance minus the clearance of another substance (X) divided by the inulin clearance equals the proportion of substance X reabsorbed in the tubules. For example: Inulin Clearance (125) minus Urea Clearance (75), divided by Inulin Clearance (125) equals 0.4, indicating that 40 per cent of the urea of the glomerular filtrate is reabsorbed during its passage through the tubules; (c) when a substance has a clearance higher than that of inulin, it is excreted partly or entirely by the tubules (p. 351). Such data have been found to be useful in studying the mode of action of diuretic agents and abnormalities in the excretion of such substances as glucose and uric acid.

A decrease in inulin clearance may result from (1) decrease in renal blood flow, (2) partial obliteration of or decrease in the number of functioning glomeruli (glomerulonephritis, glomerulosclerosis, destructive or suppurative lesions of the renal parenchyma) or (3) decrease in the effective glomerular filtration pressure. The last (p. 342) is the resultant of (a) the glomerular blood pressure, (b) the plasma colloid osmotic pressure and (c) the capsular pressure. Diminution in the first or/and increase

in the last two forces results in a decrease in effective filtration pressure. The factors that influence the filtration pressure and the clinical conditions in which it is altered are considered elsewhere (p. 342).

Determination of glomerular filtration (inulin clearance) is obviously of enormous value in investigating the pathologic physiology of urine formation. However, except under unusual circumstances, the determination of urea clearance is entirely satisfactory in the clinical study of renal function in patients with renal disease and for purposes of diagnosis and prognosis. The values for urea clearance usually parallel those for inulin clearance and the latter may usually be calculated from the former according to the following formula: $\text{Urea Clearance} / 0.6 = \text{Inulin Clearance}$. The percentage of average normal inulin clearance may be calculated by dividing the inulin clearance value by 1.25.

Renal Blood Flow—Diodrast Clearance. Diodrast is excreted largely by the tubules, *i.e.*, by transfer from the peritubular capillaries across the cells of the proximal tubule into the lumen of the tubule. It has been found¹⁸ that the removal of diodrast from the plasma is nearly complete (80-90 per cent) at low plasma concentrations of diodrast, and that the plasma diodrast concentration is approximately equivalent to the "effective" renal plasma flow, *i.e.*, the flow to active renal excretory tissue. The total "effective" renal blood flow can be calculated if the fraction of plasma present in the blood is known (hematocrit determination). Values for renal plasma flow in normal subjects have been reported ranging from about 325 to 920 cc. per minute per 1.73 sq. meters of body surface, with averages of 500-670 cc. Reported values for total renal blood flow range from 520 to 1560 cc. per minute per 1.73 sq. meters of body surface, averaging 860-1115 cc.^{15,24} Para-amino hippuric acid may be used instead of diodrast for this purpose, with identical results.

Decrease in "effective" renal blood flow (diodrast clearance) may occur as a result of: (1) generalized circulatory stasis, as in congestive heart failure and the shock syndrome; (2) local changes in the kidneys or renal circulation, *i.e.*, (a) decrease in the mass of functioning renal tissue (hypoplasia and destructive renal lesions, as tuberculosis, malignancy, suppuration, etc.), (b) decrease in the renal vascular bed (renal arteriosclerosis, arteriolosclerosis, glomerulonephritis) and (c) increased local resistance to the flow of blood resulting from constriction of the afferent or efferent glomerular arterioles. An absolute increase in renal blood flow may occur after administration of typhoid vaccine or

other pyrogenic agents.⁴⁵ Following unilateral nephrectomy the blood flow through the remaining kidney may increase as much as 70-100 per cent within a few minutes and may be temporarily increased also by a high meat diet.²⁸

Tubular Excretory Mass.⁴⁶ Inasmuch as the process of tubular excretion is limited by the mass of functioning tubular tissue available for the transfer of any substance from the blood to the urine, the measurement of the maximal rate of excretion of a substance excreted by this mechanism constitutes a measurement of the "tubular excretory mass" of the kidneys. This value, designated T_m , is calculated from simultaneous inulin and diodrast clearance determinations at high levels of plasma diodrast according to the formula,

$$T_m = U_d V - P_d C_{in} W F = \left(\frac{C_d}{C_{in}} - W F \right) P_d C_{in},$$

where U_d is the concentration of diodrast per cc. of urine, V is the urine volume in cc. per minute, P_d the quantity of diodrast per cc. of plasma, C_{in} the inulin clearance, C_d the diodrast clearance, W the fraction of water in the plasma and F the fraction of diodrast free in the plasma.⁴⁹ This value represents merely the difference between the total excretion of diodrast per minute and the quantity excreted by filtration.

It has been suggested¹⁸ that the T_m may be calculated indirectly as follows:

$$T_m = \frac{\text{Sp. Gr.} - 3.4}{4.8} \sqrt{UC},$$

where Sp. Gr. represents the 2nd and 3rd decimal place figures of the maximum urinary nonprotein specific gravity obtained by the concentration test (p. 386) (*viz.*, 1.028 = 28.0) and UC is the urea clearance in terms of per cent of average normal.

The normal diodrast T_m has been found to range from 36.6 to 72.0 mg. iodine per 1.73 sq. meters of body surface per minute, averaging 53.3 in men and 46.7 in women. The value of this determination lies in the fact that, at effective plasma diodrast concentrations and with adequate but not necessarily normal renal blood flow, the diodrast T_m is independent of glomerular activity and reflects the amount of functioning renal tubular tissue. For example, if one kidney were removed, T_m would be diminished by 50 per cent; if a portion of the excretory tissue were destroyed (either tubular destruction or obliteration of circulation), T_m would be reduced in proportion to the extent of destruction. If the glomeruli were entirely obliterated without impairing the circulation to the tubules, T_m would be unaltered.⁴⁶

The ratio, C_d/T_{md} (diodrast clearance/diodrast tubular excretory mass), is an expression of the volume of plasma com-

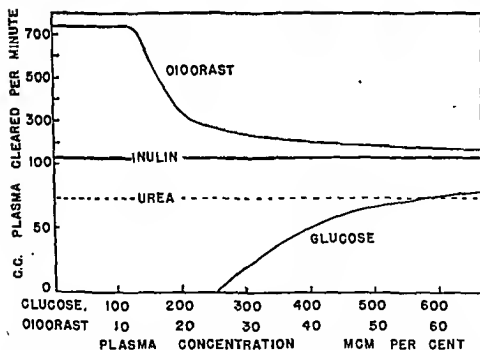
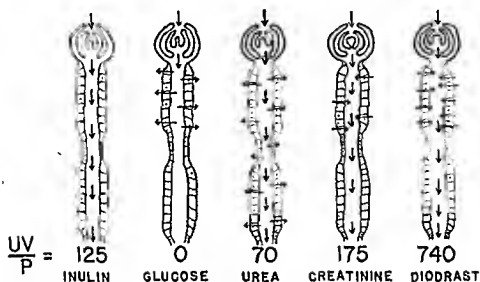


Fig. 14 —Characteristic clearance values. Scheme to illustrate mechanisms of excretion: Inulin is excreted by glomerular filtration alone, with no tubular reabsorption; Glucose is excreted by glomerular filtration but is completely reabsorbed by the tubule; Urea is excreted by glomerular filtration but is partially reabsorbed by the tubule; Creatinine is excreted by glomerular filtration and to some extent also by tubular excretion; Diodrast is excreted both by glomerular filtration and by tubular excretion. (After Smith¹⁶ and Gamble.^{17a})

pletely cleared of diodrast per unit quantity of the excretory tissue effecting this clearance. Normal values range from 10.2 to 16.7, averaging 13.4.

Tubular Reabsorption. If another substance as freely filtrable through the glomeruli as inulin has a clearance value lower than the latter, it has undergone reabsorption in the tubules. The extent of this reabsorption may be calculated as follows:

$$\text{Reabsorption} = \frac{\text{Inulin Clearance (e.g. 125) minus Clearance of X}}{\text{Inulin Clearance (125)}}$$

By means of this calculation it is evident that about 40-50 per cent of the urea present in the glomerular filtrate is normally

reabsorbed in the tubules. $\left(\frac{125 - 75}{125} = 0.4 \text{ or } 40\% \right)$

Filtration Fraction. The ratio of plasma inulin clearance (*i.e.*, volume of glomerular filtrate) to plasma diodrast clearance (*i.e.*, renal plasma flow) represents the fraction of plasma filtered through the glomeruli. Under normal conditions this is about 0.20 (*i.e.*, $\frac{1}{5}$), indicating that approximately 20 per cent of the water of the plasma flowing through the kidneys is filtered through the glomeruli into the lumen of Bowman's capsule.

Experimental studies have shown that increase or decrease in the filtration fraction is usually due to increase or decrease, respectively, in the tone of the efferent as compared to that of the afferent glomerular arteriole,²³ with consequent increase or decrease, respectively, in intraglomerular blood pressure. Renal hyperemia produced by a pyrogen is probably due to predominantly efferent arteriolar dilatation, since the renal blood flow increases and the filtration fraction decreases. The tone of the efferent arteriole is increased by administration of epinephrine and in orthostatic and psychogenic vasoconstriction,⁴⁵ with consequent decrease in renal blood flow and increase in the filtration fraction. In essential hypertension, as well as in hypertension induced by administration of renin or angiotonin (experimental), the renal blood flow (diodrast clearance) is usually decreased, glomerular filtration (inulin clearance) is often normal, and the filtration fraction is frequently increased; this combination of circumstances can be produced practically only by predominantly efferent arteriolar constriction.²⁶ Because of the increased filtration fraction, glomerular filtration and urea clearance may be maintained within normal limits until late in the course of essential hypertension (p. 378); under such circumstances, the normal urea clearance is not an expression of absolute integrity of renal function, but is usually an indication of renal vasoconstriction. An increase of filtration fraction from 0.2 to 0.3 is adequate to maintain urea clearance at 100 per cent of normal at a time when renal blood flow has fallen from a

normal level of 1000 cc. per minute to an ischemic level of 600 cc. per minute.¹⁷

Other Clearance Methods. Hippuran may be used instead of diodrast for the determination of renal plasma and total blood flow. Phenol red has been replaced by these substances for this purpose.⁴⁸ The clearance of creatinine^{41,50} and of ferrocyanide⁵¹ has been employed in the past for estimating the rate of glomerular filtration, but has been found to be inaccurate for this purpose and has been supplanted by the inulin clearance. The subject of urea clearance is discussed in detail elsewhere (p. 372).

ELIMINATION OF WATER

Comparison of Fluid Intake and Fluid Output. The quantity of water eliminated by the kidneys in twenty-four hours depends upon two factors, namely, the amount of water supplied to the body and the amount lost through extrarenal channels or otherwise rendered unavailable for excretion by the kidneys.

The organism receives water from three main sources:

(a) Ingested liquids.

(b) Ingested solids. This source of supply is too frequently overlooked. The water content of many solid foods is extremely high, and, in some instances, is higher than that of certain foods commonly considered to be liquid. For example, tomatoes contain a greater percentage of water than does milk. In any accurate study of water balance the water content of so-called "solid foods" must be taken into consideration.

(c) Water of combustion, or metabolic water. 100 Gm. of dry starch metabolized in the body form 55.5 Gm. of water, and a similar quantity of glucose, 60 Gm. of water. 100 Gm. of fat metabolized in the body form approximately 110 Gm. of water. This source of supply of water to the organism is seldom appreciated. It usually represents but a small proportion of the total supply, however, and does not introduce a serious error in the calculation of the water intake.

The kidneys ordinarily excrete more than one half of the water eliminated from the body (p. 249). Under normal conditions the remainder is lost largely through the vaporization of water in the respiratory passages and by the skin. The average quantity lost daily in this way is 700 Gm. In the presence of active perspiration or fever this amount is increased and that eliminated by the kidneys is correspondingly diminished. The amount of water excreted by normal kidneys may be greatly decreased in the presence of certain pathologic conditions which cause depletion of the water reserves of the body. Among these are severe diarrhea, vomiting, excessive expectoration, profuse

purulent discharge, biliary fistula, etc. Furthermore, prerenal deviation of water may accomplish the same result, as in myocardial weakness with edema.

All of these extrarenal factors must be taken into consideration when studying renal function on the basis of the quantitative relationship between water supply and urine volume. Ordinarily the latter is simply compared with the fluid intake. The total water supply, in individuals on an average diet, exceeds the fluid intake by about 700 Gm.; in the absence of the pathologic factors mentioned above, the water loss exceeds the urine volume by approximately the same amount. Therefore, under such circumstances, the consideration of fluid intake as total water supply and urine volume as total water elimination involves an error of approximately only 5 per cent. Interpretations based upon such observations should be made, however, with full realization of the inherent possibility of serious error, particularly in the presence of extrarenal factors which may influence the water balance.

Water Function Test (Dilution Test). This test possesses the advantage of putting a strain upon the water excretory function of the kidneys. It may be performed as follows:

(a) The fasting individual, after emptying the bladder, ingests 1200 cc. of water in one-half hour.

(b) The bladder is emptied at hourly intervals for four hours.

In the normal subject approximately 1200 cc. are eliminated within four hours, the larger part being excreted in the first two hours. The specific gravity of one of the hourly specimens should fall to at least 1.003. If renal function is impaired the quantity eliminated in four hours may be quite small and the specific gravity often 1.010 or higher, although lower figures are obtained not infrequently. Identical results may be obtained if any of the extrarenal factors mentioned above are operative. The differentiation between renal and extrarenal causes of defective water excretion must depend upon the results of other tests of renal function. The value of the dilution test lies in its clinical availability; it rarely adds any significant information to that obtained by other methods.

The ability of the kidney to eliminate water may be impaired in all types of renal disease, acute or chronic. This impairment may appear to be most pronounced in those cases associated with oliguria and edema. However, it is now recognized that failure of elimination of water is not the fundamental factor in the causation of edema. The evidence has been summarized by Marriott and Clausen as follows:

(1) Complete nephrectomy does not lead to edema.

(2) When lesions morphologically identical with those of acute Bright's disease are produced by certain toxic agents, edema does not occur.

(3) Edema may occur before there is any evidence whatsoever of renal involvement.

(4) The edema is not due to failure of elimination of salt by the kidneys.

The edema of acute or chronic nephritis and the nephrotic syndrome is probably due largely to prerenal deviation of fluid into the tissues, caused by increased capillary permeability, decrease in the colloid osmotic pressure of the blood plasma (decreased plasma albumin concentration), or increase in capillary blood pressure (congestive heart failure) (p. 258). The marked degree of oliguria which is an outstanding feature of such cases is usually largely secondary to the extrarenal factors mentioned above.

The existence of polyuria in chronic nephritis may mask a definitely-impaired ability to eliminate water. Polyuria in such cases must be regarded as a compensatory mechanism whereby the kidney, its ability to excrete solids in their normal concentration being impaired, must dilute these substances in order to secure their adequate elimination. As in the case of other organs, such as the heart, this compensation is effected at the expense of the functional reserve capacity. Therefore, when such a test as the dilution or water function test is performed and 1200 cc. of water are ingested within one-half hour, only a small fraction may be eliminated in the succeeding four hours. As Fishberg states, if such a patient can excrete only 400 cc. in the four hours, a markedly deficient water excretion, he can still maintain a polyuria of 2400 cc. in twenty-four hours. This concept of the significance of polyuria as an evidence of renal compensation for an existing deficiency in concentrating ability will be discussed further in the consideration of the unitary nature of impairment of renal function. It becomes apparent that edema and oliguria may be less indicative of primary disturbance of the excretory function of the kidney than is polyuria, paradoxical as such a statement may seem.

CHLORIDE ELIMINATION

The kidneys play an important part in the maintenance of the chloride balance of the body. Normally the organism is in chloride equilibrium, the quantity ingested being practically balanced by that excreted in the urine (average, 10-16 Gm. in twenty-four hours, as sodium chloride). This salt, existing in high concentration in all body fluids, exerts an important influence

purulent discharge, biliary fistula, etc. Furthermore, prerenal deviation of water may accomplish the same result, as in myocardial weakness with edema. •

All of these extrarenal factors must be taken into consideration when studying renal function on the basis of the quantitative relationship between water supply and urine volume. Ordinarily the latter is simply compared with the fluid intake. The total water supply, in individuals on an average diet, exceeds the fluid intake by about 700 Gm.; in the absence of the pathologic factors mentioned above, the water loss exceeds the urine volume by approximately the same amount. Therefore, under such circumstances, the consideration of fluid intake as total water supply and urine volume as total water elimination involves an error of approximately only 5 per cent. Interpretations based upon such observations should be made, however, with full realization of the inherent possibility of serious error, particularly in the presence of extrarenal factors which may influence the water balance.

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(1) Complete nephrectomy does not lead to edema.

(d) The "renal threshold" for chloride may be lowered in nephritis with renal insufficiency, thus further depleting the plasma chlorides (Peters).

(e) The chloride intake is usually low in such patients.

In view of these considerations, alteration in the plasma chloride concentration must be regarded critically and interpreted with proper appreciation of the important rôle played by extrarenal factors.

Chloride Balance. An excessive quantity of sodium chloride, ingested by a normal individual, is usually largely eliminated in the urine within twenty-four to forty-eight hours. This fact is employed as the basis for the following test of renal function:

For three days prior to the performance of the test the patient is placed upon a diet containing about 4 Gm. of sodium chloride, the exact amount being known. An extremely low salt intake is not desirable because of the production of tissue chloride deficiency with consequent retention when an excess is supplied, thus interfering with the interpretation of the results of the test. The chloride content of the urine is determined daily. On the fourth day, in addition to the previous diet, 10 Gm. of sodium chloride are administered, preferably in capsules. The excess chloride is normally eliminated within forty-eight hours, the greater part within twenty-four hours. In the presence of chloride retention the elimination is delayed and, in some cases, the quantity of chloride in the urine may be practically the same as during the control period.

Here again, extrarenal factors and prerenal deviation of chloride must be considered. This test cannot distinguish between chloride retention of renal and of extrarenal origin. It is rather disagreeable, not without danger, particularly in the presence of edema, and affords but little information regarding the state of renal function which cannot be more readily obtained by other methods.

ELIMINATION OF NONPROTEIN NITROGENOUS SUBSTANCES

The methods of study of the ability of the kidneys to eliminate the nonprotein nitrogenous end-products of protein metabolism may be classified as follows:

- (1) Urinary Studies.
- (2) Blood Studies.
- (3) Simultaneous Urinary and Blood Studies.

Urinary Studies

Studies of urinary nonprotein nitrogenous elements from the standpoint of the investigation of renal function have been

upon osmotic processes and its optimum concentration is therefore jealously preserved by the organism. It consequently constitutes one of the "threshold" substances of the urine, being eliminated normally only if its concentration in the blood exceeds the threshold value.

The study of the ability of the kidneys to excrete chloride may be approached from two angles: (1) the determination of the chloride content of blood plasma, and (2) the determination of the chloride content of the urine following the ingestion of a known quantity of sodium chloride.

Plasma Chloride (see Chloride Metabolism, p. 223). The chloride content of normal blood is 450-500 mg. per 100 cc. (as sodium chloride). The chloride content of blood plasma is 570-620 mg. per 100 cc. (as sodium chloride). This discrepancy is due to the fact that the red corpuscles contain less chloride than the plasma; therefore, in order to avoid variations dependent upon alterations in the volume and number of red corpuscles, chloride determinations should always be made upon plasma (p. 225).

Employed as a test of renal function the determination of the plasma chloride content is quite unsatisfactory. This is due to the important modifying influence of extrarenal factors which are commonly associated with renal disease. Theoretically, renal insufficiency should be associated with retention of chloride in the blood. This condition exists in certain patients with acute and chronic glomerulonephritis usually without marked edema but with severe oliguria. It is likewise observed at times in anuria due to urinary obstruction (prostatic, ureteral). Figures as high as 1000 mg. per 100 cc. have been reported although values above 750 mg. are rare. However, in most patients with nephritis and renal insufficiency the plasma chloride concentration is diminished for the following reasons:

(a) Edema fluid contains chloride, usually in slightly greater concentration than in the blood plasma. In the presence of extensive edema, particularly if the chloride intake is low and one or more of the factors listed below are operative, the deviation of large quantities of chloride to the edematous tissues may result in diminution in its concentration in the plasma.

(b) Vomiting is a common manifestation of advanced renal insufficiency and uremia. Large amounts of chloride are lost in this way and the plasma chloride is correspondingly decreased.

(c) As de Wesselow has pointed out, the acidosis constantly associated with advanced renal insufficiency causes a shift of chloride from the plasma into the red cells, thus diminishing the plasma chloride concentration (p. 237).

may be an actual preliminary fall which is believed by some to be of serious prognostic significance.

The creatinine tests of renal function do not enjoy wide popularity and rarely yield information which cannot be more readily obtained by other methods.

Urea Balance. It is evident that, because of the fact that the urea output is so directly dependent upon the protein intake, no conclusions can be drawn from the determination of urinary urea without comparing the amount excreted with the nitrogen intake. Upon an average protein intake, the urinary urea nitrogen constitutes about 80 per cent of the total urinary nitrogen; upon a high protein diet this figure rises to 90 per cent and falls to 60 per cent on an extremely low protein intake. Minor grades of retention obviously cannot be determined by this means, and an approximate nitrogen equilibrium may be maintained in the presence of moderate degrees of nitrogen retention as evidenced by an increase in the urea or total nonprotein nitrogen concentration of the blood. The most satisfactory nitrogen balance test consists in the determination of the rate of elimination of an excess of preformed urea.

The patient is placed upon a fixed diet for two days and the total nitrogen and urea nitrogen content of each day's urine is measured. The diet recommended by Strauss contains 60 Gm. of protein, 4 Gm. of salt and a fluid intake not exceeding 1500 cc. On the third day the patient is given 20 Gm. of urea dissolved in milk in addition to the same diet. The urinary nitrogen and urea nitrogen are determined in twelve-hour periods. In the absence of renal disease the added urea is eliminated within forty-eight hours, usually within thirty-six and, at times, within twenty-four hours. In the presence of renal functional impairment the urea elimination is delayed, and, in some instances, no effect is noted, the added urea being almost completely retained in the blood and tissues, particularly in the liver. As is the case in all balance tests, the true condition of renal function is not revealed during periods of compensation. For this reason the concentration tests described below are much more satisfactory from the standpoint of accurate early diagnosis.

Urea Concentration Test (Maclean and de Wesselow). This test is best performed in the morning, no food and little water being taken for the preceding twelve to eighteen hours. The bladder is emptied and the patient ingests 15 Gm. of urea dissolved in 100-150 cc. of water flavored with tincture of orange. The bladder is emptied one and two hours later and the percentage of urea determined in each specimen. If the volume of the second specimen is greater than 150 cc. a third-hour speci-

largely directed toward urea and creatinine. These studies have been essentially of two varieties: those designed to determine the ability of the kidneys to eliminate a known quantity of preformed urea or creatinine (balance studies), and those designed to determine the ability of the kidneys to concentrate urea (urea concentration tests). This type of study is not employed extensively at the present time, having been largely replaced by the more informative clearance studies.

Creatinine Balance. Many observers have noted that the urinary creatinine is low in cases of renal insufficiency and it has been suggested that, inasmuch as this factor is uninfluenced by the character of the diet, the mere determination of the creatinine elimination in twenty-four hours might afford some index of the condition of renal function. It is obvious, however, that during the stage of compensation this method of study will yield inaccurate information. A more satisfactory test consists in the determination of the rate of elimination of preformed creatinine administered by mouth or intravenously.

(1) *Oral Method (Kahn).* The test period is three successive days, during which time the urine is examined for creatinine in six-hourly periods. The first day is called the "fore-period." During this period the creatinine elimination goes on in the form of a horizontal curve, independent of the diet if meats are not taken in excess. At the beginning of the second day, the patient receives, with his diet, 1.5 Gm. of creatinine in sweetened water. A normal individual excretes 60-90 per cent within the first six-hour period after its ingestion and 8-30 per cent more in the next six hours, 70-100 per cent being eliminated within twelve hours. In the presence of renal functional impairment, creatinine elimination is delayed, the curve of excretion rising gradually.

(2) *Intravenous Method (Major).* The patient empties the bladder and the urine is collected for a one-hour period. He is then given 0.5 Gm. of creatinine intravenously and 200 cc. of water by mouth. The urine is collected promptly at the end of one hour, 200 cc. of water are taken and the urine collected again at the end of two hours (after injection). If desired, in studies of the function of each kidney by ureteral catheterization, fifteen to thirty minute periods may be employed instead of hourly periods. In the absence of renal disease there is a sharp rise in the creatinine elimination, the quantity excreted during the first period following the injection being two or three times that excreted during the control period. Patients with renal functional impairment do not exhibit this rapid rise, the curve of elimination being almost horizontal or rising gradually. There

by which the kidney can compensate for this defect is by the elimination of increased quantities of water, the urine during this period of compensation being constantly of large volume and relatively low specific gravity.

During this compensatory stage, in spite of the presence of rather advanced grades of renal functional impairment, the blood nitrogen values remain within normal limits. They rise in the absence of extrarenal factors only when the renal lesion has progressed to the point at which the kidney fails to eliminate sufficient water to compensate for the diminution in its power of concentration. Under such circumstances the urine is of consistently low specific gravity regardless of the volume of water eliminated. The variable significance of nitrogen retention dependent entirely upon renal functional insufficiency will be considered in the discussion of various types of renal disease.

Prerenal Deviation of Water. Since, particularly in the presence of renal disease, the ability of the kidneys to eliminate nonprotein nitrogenous elements depends largely upon the amount of water available for excretion by the kidneys, blood nitrogen retention may be induced by factors which cause prerenal deviation of water. The most important of these may be classified under two headings: (1) those producing edema, and (2) those resulting in dehydration.

(1) *Edema.* Water retention in the tissues, due to any cause, imposes a burden upon the nitrogen-excretory function of the kidneys, particularly if their concentrating ability is already impaired. This factor plays an important part in the production of nitrogen retention in acute nephritis and in the necrotizing nephroses (bichloride of mercury poisoning). The occurrence of myocardial insufficiency with edema, so frequently a complication of chronic hypertensive nephritis, may precipitate renal insufficiency in an individual with a previously well-compensated renal functional defect. The same phenomenon may be observed in association with edema due to a nephrotic lesion or to malnutrition with hypoproteinemia complicating chronic nephritis.

(2) *Dehydration.* Excessive loss of water or water privation exerts a similar influence. Protracted vomiting, so common a feature of advanced nephritis, and, less commonly, profuse diarrhea, may result in nitrogen retention in patients with previously compensated renal functional impairment. Excessive perspiration or vaporization of water from the body surface in complicating febrile disorders may act in a similar manner.

Excessive Protein Catabolism. With a constant state of renal functional activity, an excessive rate of protein catabolism will

men is collected. Normally the concentration of urica is 2 per cent or more in one of the specimens, usually the second. Mild impairment of function is indicated by a concentration of 1.8 per cent and more severe grades by concentrations below 1.6 per cent.

This procedure is extensively employed in England and unquestionably yields valuable information during both compensated and uncompensated stages of renal disease. However, it frequently fails to denote accurately the degree of renal damage and at times may be misleading. In some patients with nonrenal edema, the diuretic action of urea may result in its elimination in relatively low concentration. In some cases of advanced renal disease with edema, values of 1.8 to 2 per cent are occasionally obtained. The test is, however, valuable and is correct in principle, since it attempts to measure a most important function of the kidney, that of concentration of urea.

Blood Nitrogen Studies

The several conditions which may be associated with elevation of the level of nonprotein nitrogen in the blood have been reviewed previously (p. 100). The following discussion is concerned only with the problem of blood nitrogen retention in its relation to renal functional impairment.

In the presence of renal disease, blood nitrogen retention depends essentially upon three factors:

- (a) The degree of renal functional impairment.
- (b) Prerenal deviation of water.
- (c) Excessive protein catabolism.

Since the diagnostic and prognostic significance of nitrogen retention depends upon the part played by each of these factors in any given case, they will be considered individually in greater detail.

Renal Functional Impairment. The concentration of non-protein nitrogenous substances in the urine greatly exceeds that in the blood. Under ordinary conditions the concentration of urea in the urine is 65 times as great as in the blood, uric acid 30 and creatinine 35 times as great. The ability to concentrate these and other solid substances constitutes one of the most important, if not the most important, function of the kidney. In view of its capacity in this direction the normal kidney can satisfactorily eliminate the required quantity of solids even though only relatively small quantities of water are available for their solution. Inability of the kidney adequately to eliminate nitrogenous substances may be regarded as dependent essentially upon diminution in its concentrating ability. The only method

normally approximately one half of the total nonprotein nitrogen of the blood, usually constitutes by far the greater proportion of elevated blood nitrogen values. In individuals with 200 mg. per 100 cc. of nonprotein nitrogen, urea nitrogen may constitute 80-90 per cent of the total. In some instances, however, the undetermined fraction may be increased relatively more than urea, although such findings are uncommon.

Uric Acid. Myers, Fine and Lough made the observation that, in the presence of renal failure, the uric acid concentration in the blood increases before that of urea and creatinine. They inferred from this observation that uric acid is normally eliminated with less facility than are the other nitrogenous elements and hence is the first to be retained in the blood under conditions of renal functional impairment. This hypothesis has not received general confirmation, and, although the blood uric acid is usually increased in renal insufficiency, it is far less satisfactory as an index of the degree of functional impairment or even of the presence of renal disease than is the blood urea concentration. Patients with gout, arterial hypertension and other conditions (see pp. 103ff.) may exhibit elevations in blood uric acid in the absence of renal disease.

Creatinine. Myers and Lough believed that creatinine, being readily eliminated by the kidney, increases in concentration in the blood only when renal function is seriously impaired and hence creatinine retention has a serious prognostic significance (normal 1-2 mg. per 100 cc.). Whereas it is commonly observed that in renal insufficiency the blood urea concentration may be considerably increased for long periods of time before any significant increase in creatinine occurs, the explanation advanced by Myers and his associates has not been generally substantiated. As stated by Fishberg, it may well be that the increase in blood urea in the presence of normal creatinine is due to extrarenal causes rather than to dissociated injury to individual functions of the kidney and that the problem involved may be one of differences in supply rather than of excretion. There can be no question, however, of the grave import of high blood creatinine values. The variable significance of creatinine retention in various types of renal disease will be considered below.

Ammonia, Amino Acids, Undetermined Nitrogen. Blood ammonia is not increased in renal insufficiency, nor are amino acids, in most instances. Occasionally, however, the amino acid content of the blood may rise in the late stages of uremia, due perhaps to toxic protein catabolism or to acute hepatic necrosis, which may occur in that condition. The undetermined nitrogen fraction is usually elevated in proportion to the increase in total

cause a tendency toward the accumulation of nitrogenous elements in the blood. This factor may play a not inconsiderable part in the development of nitrogen retention in nephritis. Among the more common conditions associated with increased protein destruction and a consequent increase in the quantity of nonprotein nitrogenous substances that must be eliminated by the kidneys are the following:

(1) *High Protein Intake.* Under conditions of normal renal function, normal water balance and adequate fluid intake the ingestion of large quantities of protein is followed by prompt elimination of the excess nitrogen with no consequent significant elevation of the level of nitrogen in the blood. However, if renal function is impaired, and especially if, in addition, the urine volume is diminished, the nonprotein nitrogenous elements in the blood may increase during periods of high protein intake and return to normal following the administration of a diet low in protein.

(2) *Infections.* Infectious processes, occurring in patients with renal functional impairment, may result in an increase in blood nitrogen. Infections such as tuberculosis, even in the absence of fever, cause toxic destruction of protein. In febrile disorders, increased protein catabolism results from both toxic destruction and increased energy requirement incident to the elevation of body temperature. The tendency toward nitrogen retention in such conditions is aggravated by the frequent coexistence of other factors exerting a similar influence, such as deficient fluid intake, excessive evaporation from the skin and respiratory tract, diarrhea, vomiting, edema, and so on. Excessive protein catabolism may perhaps contribute to the nitrogen retention frequently observed in acute nephritis and bichloride nephrosis.

(3) *Dehydration.* Dehydration due to water privation, vomiting, diarrhea, or the like, so alters tissue metabolism as to result in an increase in the rate of protein catabolism, a phenomenon commonly regarded as toxic destruction of protein. The increase in the level of blood nitrogen which occurs under such circumstances is due to the combined effects of this factor, diminution in the quantity of water available for elimination by the kidneys and inadequate renal blood flow.

Obviously, the interpretation of the significance of blood nitrogen retention in individuals with renal disease depends upon the proper evaluation of the relative importance of renal and extrarenal factors in its production.

Relative Degree of Retention of Individual Nitrogenous Elements in Renal Disease. *Urea.* Urea nitrogen, representing

This prerenal deviation of water is one of the most important factors in determining the presence or degree of blood nitrogen retention in acute nephritis. Occasionally edema due to myocardial insufficiency may exert an influence in this connection as may, more frequently, limitation of fluid intake and active purgation, which are common practices in the presence of edema.

(3) EXCESSIVE PROTEIN CATABOLISM. Acute nephritis should be considered as merely a local manifestation of a generalized process affecting the tissues and capillaries throughout the body. It is consequently associated with increased protein catabolism due to both toxic protein destruction and increased protein requirement. In fact this feature may be so pronounced that an increase in urinary nitrogen and a negative nitrogen balance may exist in association with an increase in the nonprotein nitrogen content of the blood. Relatively high grades of creatinine retention in acute nephritis may be an expression of excessive endogenous protein metabolism.

It is evident, therefore, that several factors must be considered in determining the prognostic significance of high blood nitrogen values in acute nephritis. In most cases the condition is a temporary one, the nitrogen values returning to normal upon subsidence of the acute infection. Occasionally, with improvement in the clinical manifestations, a transient rise in blood nitrogen may occur during periods of diuresis and diminution in edema. Nitrogen retention associated with urine of a low specific gravity, indicating renal functional impairment, is much more significant than that associated with urine of a high specific gravity. Because of the essential character of the condition, with its inherent capabilities of retrogression and eventual recovery, anatomical and functional, high nitrogen values have relatively little prognostic significance in acute nephritis. This is particularly true if they are dependent largely upon extrarenal factors as is commonly the case. Creatinine retention has not the serious significance attributed to it in chronic renal disease and extremely high values, although not to be disregarded, are not incompatible with complete recovery.

The proper interpretation of the retention of nonprotein nitrogen in the blood in acute, as indeed in chronic nephritis, depends upon the relative part played by the renal lesion per se, whether it subsides or progresses into chronic nephritis, and upon the relative importance of extrarenal factors. Single determinations are of little value because of marked temporary variations due to extrarenal influences. In the majority of cases nitrogen retention may be regarded as a result of the combined

nonprotein nitrogen. In a few instances, however, relatively high values have been observed in uremia. The significance of this fraction has not been determined.

Blood Nonprotein Nitrogen in Renal Disease. Nitrogen retention in renal disease depends upon the nature and extent of the renal lesion and upon extrarenal factors, chiefly prerenal deviation of water and excessive protein catabolism. Since the relative importance of each of these factors may vary in different types of renal disease, the significance of blood nitrogen retention must vary accordingly.

Acute Glomerulonephritis. Increased blood nitrogen values are frequently observed in acute nephritis, the degree of retention varying from slight elevations (N.P.N. 45 mg., Urea N 25 mg., Creatinine 2 mg. per 100 cc.) to extremely high figures (N.P.N. 200 mg., Urea N 160 mg., Creatinine 25 mg. per 100 cc.). Nitrogen retention in this condition is the resultant of several factors:

(1) **IMPAIRED RENAL FUNCTION.** There is considerable variation in the degree of impairment of renal function in acute nephritis. In many cases renal function is intact, as evidenced by the ability of the kidney to concentrate the solid constituents of the urine (high specific gravity). One does not usually observe marked evidences of advanced renal insufficiency due to the renal lesion per se (oliguria with low specific gravity) in this type of nephritis. Many cases in which this occurs are in reality instances of an acute exacerbation of chronic nephritis. However, in the majority of such individuals there is slight or moderate renal functional impairment. This, in itself, is not always sufficient to cause the marked elevations of blood nitrogen which are frequently observed and which are dependent upon the operation of extrarenal factors superimposed upon a mild renal functional defect.

(2) **PRERENAL DEVIATION OF WATER.** Oliguria is an important feature of acute nephritis. Whereas in a relatively small proportion of cases this is a manifestation of renal functional impairment (glomerular occlusion), it is usually dependent upon prerenal deviation of water, due to edema, vomiting or fever with increased vaporization of water from the skin. The edema of acute nephritis is probably not dependent upon the renal lesion per se but rather upon generalized capillary injury with consequent increased permeability, upon a diminution in the concentration of plasma proteins, or upon myocardial weakness (p. 258). Edema may be latent or frank; as much as 4000 cc. of water may be retained without being detectable by ordinary methods of physical examination.

factors. Under such circumstances, being not necessarily indicative of the actual extent of the renal lesion, nitrogen retention loses its serious significance. As a rule, the course of the disease is punctuated by periods of excessively high blood nitrogen values which subside after correction of extrarenal influences. Among the most common of these are intercurrent infections, excessive vomiting and diarrhea, excessive protein ingestion, water privation, acute exacerbations of the renal process, and myocardial failure with consequent edema. These factors have been considered in detail above; they operate largely in one or both of two ways, *i.e.*, increased protein catabolism and prerenal deviation of water. Thus renal insufficiency is precipitated by the imposition of an excessive burden upon a diminished renal functional reserve. Upon removal or cessation of operation of these factors the blood nitrogen concentration falls to the level determined by the degree of renal functional impairment and the efficiency of the compensating mechanism (polyuria).

It must be apparent that too much reliance must not be placed upon the concentration of the nonprotein nitrogenous constituents of the blood as an indication of the extent of renal damage in chronic nephritis. In the absence of complicating conditions the pathologic process in the kidneys may be far advanced before nitrogen retention occurs; in the late or terminal stages it may have some prognostic significance, particularly if creatinine retention is marked. However, at any time during the course of the disease advanced grades of nitrogen retention may occur because of the operation of one or more of the several extrarenal factors mentioned above. In the interpretation of high nonprotein nitrogen values these complicating influences must be carefully considered and their part in the production of the existing condition properly evaluated.

Nephrosclerosis. The concentration of nonprotein nitrogen in the blood is usually normal during the entire course of essential hypertension and nephrosclerosis, as may be the urea clearance. Nevertheless, even in early stages, the presence of abnormality of renal function (glomerular dynamics) is evidenced by impairment of concentrating power and, frequently, reduction in renal blood flow (p. 351). In the late stages of this condition, and in so-called "malignant hypertension," renal failure may occur and progress rapidly to a fatal termination. Because of the frequent occurrence of vascular accidents or cardiac failure incident to the prolonged state of hypertension, renal insufficiency is observed less frequently than in chronic glomerulonephritis. However, if it does occur, the metabolic

effects of mild or moderate renal functional impairment, pre-renal deviation of water and excessive protein catabolism, the last two factors being usually more important than the first; hence even advanced grades of nitrogen retention in acute nephritis possess relatively little significance from the standpoint of the estimation of the extent of renal damage. They must be interpreted only in the light of information afforded by other tests of renal functional efficiency, particularly the concentration and clearance tests, which are relatively uninfluenced by extrarenal factors.

Chronic Glomerulonephritis. In the absence of extrarenal influences, nitrogen retention occurs in chronic nephritis only when the renal lesion has become far advanced. The factors involved are essentially the same as those discussed in connection with acute nephritis with the significant exception that the pathologic process in the kidneys is chronic and progressive and that, as a result, renal functional impairment plays a more important and extrarenal factors a less important part than in acute nephritis. For a variable period of time, depending upon the rapidity of progression of the renal lesion, the blood non-protein nitrogen remains within normal limits. This is due to the efficiency of the compensatory mechanism which consists in increased water excretion by means of which the existing diminution in concentrating ability is counterbalanced and adequate elimination of solids is ensured.

As the severity of the kidney lesion increases and renal functional efficiency becomes more distinctly impaired, a point is reached where increased water elimination is no longer able to compensate for the increasing inability to excrete solids. Retention of nonprotein nitrogen ensues and, at first mild, progresses steadily but with variable rapidity until in the terminal stages (uremia) extremely high levels may be reached. In patients suffering with this form of the disease, nonprotein nitrogen values of more than 100 mg. per 100 cc., urea nitrogen of 80 mg. and creatinine of 5 mg. per 100 cc. foreshadow a rather speedy fatal termination. Urea usually constitutes the greater proportion of this increase in nonprotein nitrogen (60-90 per cent), the blood creatinine commonly remaining within normal limits for a considerable period of time during which the urea nitrogen concentration has exhibited a progressive increase. The reasons for this apparent dissociation of function have been discussed previously (p. 365).

As in the case of acute nephritis, marked elevations of blood nitrogen may and frequently do occur during the course of chronic nephritis due to the superimposition of extrarenal

frequently remains normal in the presence of extremely high urea nitrogen values, in contradistinction to the findings with similar grades of urea nitrogen retention in nephritis. In the experience of the authors this is not generally the case. In fact the reverse is apparently more commonly observed; *i.e.*, with equal grades of urea retention, blood creatinine values are higher in urinary obstruction than in nephritis. This is exactly what would be expected in view of the purely mechanical nature of the condition.

In the absence of permanent renal disease, relief of the obstruction and institution of adequate drainage are usually followed by restoration of the normal blood nitrogen level. This may occur in the presence of extremely high nitrogen values. In a patient with prostatic obstruction, with a total blood nonprotein nitrogen concentration of 420 mg. per 100 cc. and creatinine 32 mg. per 100 cc., following prostatectomy in two stages the nonprotein nitrogen fell to 54.8 mg. and the creatinine to 2.6 mg. per 100 cc. Nitrogen retention in urinary obstruction does not possess the significance attributed to it in primary kidney disease.

The Urea Ratio. Mosenthal and Bruger have proposed the use of the urea ratio as an index of renal function.³⁰ This ratio is calculated according to the formula,

$$\frac{100 \times \text{blood urea } N}{\text{Blood NPN}}$$

Normal renal function is indicated by values of 44 or less, while values above 80 are obtained with maximum impairment of renal function. It has been found that the ratio rises and falls as renal function fails or improves. The obvious advantages of this procedure are that no urine analysis is required and only one specimen of blood is necessary. This procedure may be useful in cases in which the performance of the urea clearance test is not possible, but the latter is to be preferred for more accurate quantitative measurement of renal functional impairment.

Simultaneous Study of Blood and Urine

Urea Excretion Ratio of Addis. Addis and Watanabe (1916) suggested that the ratio between the rate of urea excretion and the blood urea concentration should constitute an accurate index of the functional efficiency of the kidneys. This factor, termed the urea excretion ratio, is expressed as follows:

$$\text{Ratio, } \frac{D}{B} = \frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 cc. blood}} = 50 \text{ (average normal)}$$

features and blood chemical findings are identical with those which characterize the latter condition.

Destructive Renal Lesions. Under this heading may be considered such conditions as renal tuberculosis, malignancy, pyonephrosis, pyelonephritis, hydronephrosis, polycystic kidney, and so on. The estimation of renal functional efficiency in these disorders is of particular importance from the standpoint of the advisability of surgical intervention and the determination of the operative procedure to be employed.

Since adequate elimination may be carried on by two thirds of one kidney, the presence of nitrogen retention in destructive lesions of the kidney indicates extensive renal damage. It may be interpreted as signifying either that both kidneys are involved in the pathologic process or that the uninvolved kidney is overburdened by the necessity of eliminating excessive quantities of nitrogen resulting from increased toxic destruction of protein. In this group of disorders the influence of extrarenal factors must be carefully evaluated. High protein intake, fever, water privation, vomiting, edema, diarrhea, etc., must be taken into consideration.

The consensus is that little information of significance in prognosis can be gained from single nonprotein nitrogen determinations. In destructive renal lesions it is the persistence rather than the degree of nitrogen retention to which attention should be directed and by which the extent of renal damage should be estimated. The persistence of elevated blood nonprotein nitrogen values in such cases following the institution of proper therapeutic procedures, medical and surgical, is of definitely grave prognostic import. Because of the fact that individuals with marked nitrogen retention are poor operative risks, repeated preoperative estimations of the nonprotein nitrogen concentration of the blood are of value in determining the time at which radical surgical measures may be attempted with a minimum of risk to the patient. In surgical disorders of the kidneys, the functional efficiency of each kidney should be studied by means of the estimation of its capacity for dye elimination (p. 381), determined either by ureteral catheterization or by direct vision through the cystoscope.

Urinary Obstruction. In patients with prostatic or urethral obstruction or with bilateral ureteral calculus, and in some cases of unilateral ureteral calculus (with "reflex" anuria) the back pressure may be so great that effective filtration through the glomerular membrane cannot occur. Under such circumstances remarkable grades of nitrogen retention may be observed. It is believed by some that in these conditions the blood creatinine

in 75 cc. of plasma. A more exact definition of blood urea clearance would be "the number of cubic centimeters of plasma which contain the amount of urea removed per minute by renal excretion."

It was shown that, in normal adults, when the excretion of urine proceeds at a rate of 2 cc. or more per minute, a certain volume of blood will be freed of urea each minute. This volume of blood, termed the "maximum clearance" (C_m), normally ranges from 64 to 99 cc., the mean value for an adult of average size (surface area of 1.73 square meters) being 75 cc. of blood per minute. Expressed as a formula,

$$C_m = \frac{UV}{B}$$

where U designates the milligrams of urea in 100 cc. of urine, B the milligrams of urea in 100 cc. of blood and V the urine volume in cubic centimeters per minute.

Similarly, when the urine output is below the augmentation limit (less than 2 cc. per minute), a certain volume of blood will be freed of urea each minute. This volume of blood is termed the "standard clearance" (C_s) and varies normally from 41 to 65 cc., the mean value for an adult (surface area 1.73 square meters) being 54 cc. of blood per minute. Expressed as a formula,

$$C_s = \frac{U}{B} \sqrt{V}.$$

Because of the observation that the blood urea clearance, urine volume and augmentation limit vary directly with variations in the body surface area, a correction factor should be introduced into the above formula in the case of individuals distinctly above or below the average adult size. As stated by the authors, no correction need be made for persons between 62 and 71 inches in height since the error involved does not exceed 5 per cent. The corrected formulae are expressed as follows:

$$C_m = \frac{U}{B} \times V \times \frac{1.73}{A}$$

$$C_s = \frac{U}{B} \sqrt{V \times \frac{1.73}{A}}$$

where A is the body surface in square meters, calculated according to the height and ideal weight, available in standard tables provided for such determinations.

Procedure (Möller, McIntosh and Van Slyke). The routine procedure advocated by the originators of this test is as follows:

Addis believed that this ratio is independent of the urine volume, but, as was later demonstrated by Austin, Stillman and Van Slyke, this is true only when the rate of urine excretion exceeds 2 cc. per minute (augmentation limit). If a urine volume in excess of this quantity is ensured under the conditions of the test, as was suggested by Addis, this ratio is a valuable index of renal functional efficiency. As Addis later stated (1928), it is only through recognizing that the ratio in reality represents the volume of blood freed from urea per unit of time that it may be thought of as a concrete and reasonable measure of renal function.

It was later pointed out by Austin, Stillman, Van Slyke and their associates that the urea excretion ratio of Addis was identical in principle, and, with minor mathematical corrections, identical in fact with their "maximum blood urea clearance" values. Since, in the opinion of the authors, the determination of blood urea clearance possesses a wider field of clinical application, the procedure suggested by Addis will not be discussed in further detail. It is essentially the same as that to be considered below.

Blood Urea Clearance. As a result of investigations of the nature described above it became evident that the most exact information regarding the urea excreting ability of the kidney requires comparison of the blood urea concentration and the urea excretion in the urine. It had been shown that with fairly large urine volumes the rate of elimination of urea is directly proportional to the blood urea content. Austin, Stillman and Van Slyke demonstrated that this direct relationship holds only when the urine volume exceeds a certain limit, 2 cc. per minute (adults), which they designated the "augmentation limit." With urine volumes below this figure the rate of urea elimination was found to fall, and was found to be, on the average, proportional to the square root of the urine volume. Further investigations by Möller, McIntosh and Van Slyke and their collaborators indicated that perhaps the most satisfactory medium of expression of the relationship between the blood urea concentration and the urinary urea excretion was by means of the "blood urea clearance" which is expressed as the number of cubic centimeters of blood completely cleared of urea by renal excretion. The blood of course is not completely cleared of urea in its passage through the glomerulus. According to Van Slyke,⁵³ about 10 per cent of the blood urea is so removed. Therefore, if, under conditions of maximum blood urea clearance, about 750 cc. of blood plasma pass through the kidney per minute, the amount of urea removed would be equivalent to that contained

$$C_m = \frac{U}{B} \times V = \frac{210}{14} \times 3 = 45 \text{ cc. of blood cleared of urea per minute.}$$

$$\text{Percentage of normal function} = \frac{45}{75} \times 100 = 60 \text{ per cent.}$$

Example of Calculation of Standard Clearance

Blood urea $N = 14$ mg. per 100 cc. $= B$

Urine urea $N = 420$ mg. per 100 cc. $= U$

Urine volume $= 90$ cc. per hour

$= 1.5$ cc. per minute $= V$

$$C_s = \frac{U}{B} \sqrt{V} = \frac{420}{14} \times 1.22 = 36.6 \text{ cc. of blood cleared of urea per minute.}$$

$$\text{Percentage of normal function} = \frac{36.6}{54} \times 100 = 67.7 \text{ per cent.}$$

Interpretation. Addis and Drury found that the maximum clearance is increased by the ingestion of caffeine, milk, a mixed meal and by small doses of epinephrine. It is decreased by the administration of pituitrin or large doses of epinephrine. MacKay, in a study of the diurnal variation of the standard blood clearance, found it to be depressed during the first hour after arising; following this, commencing before breakfast, there was a regular increase, the higher level continuing through the morning. A definite drop occurred after lunch with a subsequent rise during the late afternoon and evening. The least variation occurred during the period between breakfast and lunch (9-12 A. M.). It has been found that strenuous exercise causes a decrease in blood urea clearance, particularly in patients with impaired renal function.

The urea clearance may be abnormally low during periods of subsistence on a low protein diet.^{16,25} Low values have been reported during relapse in pernicious anemia and in other severe anemias.^{5,23} Increase in urea clearance has been reported following administration of large doses of vitamin A;²⁹ this may be due to some constituent of fish liver oil other than vitamin A. The urea clearance in pregnancy appears to remain within normal limits in subjects without severe anemia and with an adequate protein intake.^{6,13} Reports of low values are probably due to low protein intake.¹⁰ Normal values are the rule in eclampsia and pre-eclampsia.¹³

It has been observed that, particularly in patients under forty years of age, marked elevation of blood urea clearance (over 25 per cent above the average normal) may occur during the

The subject is not subjected to any previous routine, except that vigorous exercise is avoided. The most desirable time of day is in the hours between breakfast and lunch, at which time excretion is least liable to fluctuation. Two or three glasses of water are taken shortly before breakfast. The bladder is emptied completely, the exact time being noted. This urine is discarded. This marks the beginning of the test period, breakfast being taken at this time. Exactly one hour later the bladder is again completely emptied, the urine being saved for examination. At this time a specimen of blood is withdrawn for urea determination. Exactly one hour later the bladder is again completely emptied, the urine being saved for examination. If the urine specimens are not obtained exactly one hour apart, the exact time at which they are obtained should be noted. The volume of each specimen is measured carefully and determinations are made of the concentration of urea in the blood and urine specimens. Inasmuch as the urea is of course removed only from the plasma in the process of urine formation, plasma and not whole blood should be used for clearance determinations. However, urea exists in the water of the erythrocytes in practically the same concentration as in the plasma, so that for practical purposes in clinical studies whole blood may be employed in estimating urea clearance (but not inulin, diodrast, hippuran and other clearances). The patient remains quiet during the period of the test. The chief source of error lies in the possibility of incomplete emptying of the bladder, the liability of which is diminished by the collection of two urine specimens. In cases in which conditions are present which interfere with complete emptying of the bladder, such as prostatic hypertrophy, tumors of the uterus, advanced pregnancy, etc., the bladder should be emptied by catheter at the beginning and end of the two-hour test period.

If the urine volume (corrected) exceeds 2 cc. per minute the maximum clearance is calculated. If the urine volume (corrected) is less than 2 cc. per minute the standard clearance is calculated.

Results are expressed in terms of cubic centimeters of blood cleared of urea per minute or in terms of percentage of average normal clearance.

Example of Calculation of Maximum Clearance

$$\begin{aligned}
 \text{Blood urea } N &= 14 \text{ mg. per 100 cc.} = B \\
 \text{Urine urea } U &= 210 \text{ mg. per 100 cc.} = U \\
 \text{Urine volume} &= 180 \text{ cc. per hour} \\
 &= 3 \text{ cc. per minute} = V
 \end{aligned}$$

of protein in the space within or without this membrane. The importance of this factor in renal disease is questionable. (2) Decreased blood pressure in the glomerular capillaries. This might conceivably result from a drop in the general arterial pressure, from constriction of the afferent arterioles to the glomeruli or from dilatation of the efferent arterioles. A decrease in general arterial pressure below about 40 mm. Hg results in anuria, presumably because the glomerular blood pressure is then too low to produce an effective filtration pressure against the opposing forces of the colloid osmotic pressure of the blood and the intracapsular pressure in Bowman's capsule. A drop in urea clearance may accompany the falling blood pressure of acute cardiac failure and the shock syndrome. (3) Increased back pressure on glomerular filtrate. This occurs when the urinary flow is obstructed by lesions of the bladder or the ureter or by closure of the tubules with casts or swollen epithelium. This factor may be of importance in the renal functional impairment which may occur in amyloid disease of the kidneys and in multiple myeloma. However, moderately increased back pressure may diminish urea clearance chiefly by increasing reabsorption in the tubules. (4) Increased plasma protein concentration. If the plasma protein concentration, and consequently the colloid osmotic pressure of the blood, increases sufficiently it may effectively diminish the volume of glomerular filtrate if it produces a significant diminution in the effective filtration pressure. Vice versa, decrease in the plasma protein concentration might increase the volume of glomerular filtrate. The practical importance of this factor is questionable.

(b) Increased reabsorption of urea in the tubules. It has been found experimentally that urea may pass back from the renal tubules into the renal blood temporarily to such an extent that the renal venous blood urea concentration may exceed the arterial. Occasionally, evidence of excessive reabsorption of urea has been obtained clinically, particularly in cases of urinary obstruction. This factor plays little if any part in the production of diminished urea clearance in renal disease since, in degenerative types of renal disease, with extensive tubular damage, the clearance does not appear to be diminished unless the glomeruli are also involved.

The determination of the blood urea clearance constitutes one of the most valuable means of estimating, quantitatively, the degree of renal functional impairment during the stage of renal compensation as well as in renal insufficiency. The following data are derived largely from the work of Van Slyke and his co-workers.^{52, 53}

course of acute infections such as pneumonia and rheumatic fever.²⁰ This increase may persist for a few weeks after the temperature has returned to normal. The cause of this increase is not known, but the absence of this phenomenon in older patients is attributed to a decrease of renal resiliency with increasing age. An increase has been observed also after injection of pyrogens,⁴⁶ but not in hyperthermia induced by diathermy.³⁷ It is important also that erroneously low values may be obtained when the urine volume is less than 0.35 cc. per minute and the usual formula is applied.¹⁴ Unilateral nephrectomy is followed by an average increase of 43 per cent in urea clearance by the remaining kidney.⁵³

Abnormal decrease in the volume of blood cleared of urea per minute is due to one or both of two causes: (1) diminution in the minute volume of blood flow through the kidneys without simultaneous increase in the filtration fraction (p. 354), or (2) diminution in the proportion of urea removed from the blood during its passage through the kidneys. Van Slyke outlines the causes of diminished urea clearance as follows:^{52,53}

1. Diminished Renal Blood Flow, which may result from:

(a) Closure of an unusually large proportion of the glomerular capillaries by overstimulation of the normal function noted by Richards.

(b) Functional constriction of the renal arteries or arterioles, the angiospastic constriction regarded by some as the immediate cause of renal damage in acute nephritis. This process may be reversible.

(c) Passive congestion, in heart failure or in shock. This process may be reversible.

(d) Anatomical occlusion of arterial lumina. This occurs in advanced renal disease, whether the initial stages were of the inflammatory or the sclerotic type. This process is irreversible.

(e) Destructive occlusion of glomerular capillaries. This occurs in advanced renal disease of sclerotic and inflammatory varieties. This process is irreversible. It has been found that in nephritis the decrease in urea clearance parallels the diminution in the number of perfusable glomeruli.

2. Decreased Proportion of Urea Removed from the Blood. This may result from the following causes, all of which appear to be possibly reversible:

(a) Decrease in the volume of glomerular filtrate, which may result from the following causes: (1) Decreased permeability of the glomerular capillaries. This might result conceivably from thickening of the capillary walls or of the capsular membrane which surrounds the capillary tuft, or from the accumulation

onephthalein excretion, urine urea concentration and the like. The blood urea clearance usually falls below 50 per cent of normal before these tests yield abnormal results. Such procedures as the two-hour specific gravity fixation test and other tests of the concentrating ability of the kidneys compare favorably with it from the point of view of early diagnosis of renal functional impairment, but do not afford as exact information from a quantitative standpoint. The blood urea clearance determination constitutes the most sensitive method available at present for the estimation of the degree of renal functional damage. This general statement cannot, however, be applied without qualification. In many patients with essential hypertension, even in the presence of nephrosclerosis, the urea clearance may be within normal limits while the concentrating power of the kidneys is usually impaired. This phenomenon is due to the abnormality of renal hemodynamics in this condition, *i.e.*, predominantly efferent glomerular arteriolar constriction, increased intraglomerular blood pressure and increased extraction of water and urea from a frequently diminished volume of plasma.^{18,46} The clearance of urea may therefore be normal despite a 30-40 per cent reduction of renal blood flow (p. 351) and a reduction of renal tubular mass (p. 352). The maintenance of a normal filtration rate by the increased glomerular blood pressure results in the entrance of an abnormally large volume of filtrate into each unit of tubular mass, with decreased reabsorption of water and consequent lowering of the maximum attainable specific gravity. The maintenance of normal urea clearance under such circumstances is an indication of abnormality of renal hemodynamics.

In a comparative study of concentration tests and blood urea clearance determinations in nephritis, Alving arrived at the following conclusions:

(1) Concentration tests, performed with correction for the influence of urinary protein on the specific gravity are sensitive criteria for the qualitative detection of impaired renal function. The so-called Mosenthal test yielded similar results.

(2) Concentration tests do not appear, however, to be suitable for estimating the extent of renal damage. In some cases, disagreement between the blood urea clearance and the results of concentration tests may be extreme, particularly during the stage of recovery from acute glomerulonephritis and in active chronic glomerulonephritis. Alving states that a patient who has practically recovered from acute nephritis and has regained a normal urea clearance may yield maximum urine concentration values of 1.009 to 1.012 specific gravity, similar to those found in patients in the terminal stages of uremia with only 3-5 per cent

ACUTE NEPHRITIS. In the great majority of cases of acute nephritis the blood urea clearance diminishes during the first two months, reaching values of 50 per cent or less of normal. Occasionally, however, normal values may be maintained throughout the acute stage of the disease. Prognosis as to eventual recovery appears to depend, not so much upon the absolute values observed during the early stage, as upon the duration of the period of impaired renal function. It has been observed that recovery occurs in those cases in which a consistent rise in blood urea clearance values, progressing to within normal limits, begins within four months after the acute onset of the condition. In those cases in which no such tendency is noted within six months, the condition almost invariably progresses to the chronic or terminal stages of nephritis with eventual renal insufficiency.

Low values during the first four months of acute glomerulonephritis are not at all inconsistent with complete recovery, both functional and anatomical. During this period, therefore, the urea clearance is of little value in determining the eventual outcome of the condition, since progression into the chronic active stage of the disease may occur in cases with normal clearance values during the acute stage of the condition.

CHRONIC GLOMERULONEPHRITIS. Normal values may be obtained during the latent stage of glomerulonephritis, renal function being normal. In the chronic active stage subnormal values are always found, being consistently less than 60 per cent and usually less than 50 per cent of normal. In the terminal stage, during which blood nitrogen retention occurs, the clearance values are markedly diminished, being usually below 20 per cent of normal. Uremia was consistently present with figures of 5 per cent or less of normal.

NEPHROSCLEROSIS. Normal blood urea clearance values may be obtained for many years in patients with essential hypertension or arteriosclerotic Bright's disease. Usually, however, a gradual, slow fall in clearance occurs, which, if the patient does not succumb to the effects of cardiovascular disease, eventuates in renal failure as in the case of glomerulonephritis. Renal insufficiency, as evidenced by a marked decrease in urea clearance, may occur rather suddenly, particularly in young individuals with essential hypertension (malignant hypertension).

The blood urea clearance usually shows evidence of diminution in renal functional efficiency sooner than do most of the commonly employed functional tests, such as the determination of blood nonprotein nitrogen, urea and creatinine, phenolsul-

content of the saliva more closely approximates the concentration of urea nitrogen in the blood because of the fact that urea in the saliva is rather readily broken down into ammonium carbonate through bacterial action. Because of the mercury binding capacity of urea, Hensch and Aldrich have advocated the determination of the mercury combining power of saliva as an index of the degree of renal functional insufficiency in cases in which determination of the blood urea nitrogen concentration is not practicable. They have found that 100 cc. of saliva normally contain enough urea to combine with 30-50 cc. of a 5 per cent solution of mercuric chloride. This constitutes the mercury combining index or the salivary index. It is stated that the probable blood urea concentration may be roughly calculated from the salivary index as follows:

$$1.43 \times \text{salivary index} - 34 = \text{probable blood urea in mg. per 100 cc. of blood.}$$

The authors state that when the salivary index is below 50 there is no retention of urea nitrogen in the blood in 90 per cent of cases; in 10 per cent there may possibly be a mild degree of retention but the blood urea concentration is never above 60 mg. per 100 cc. under such circumstances. This method is by no means accurate and should be used only as a preliminary diagnostic procedure and never as a substitute for the determination of blood urea nitrogen.

THE ELIMINATION OF FOREIGN SUBSTANCES

The kidney normally possesses the ability to eliminate certain foreign substances from the blood stream. This fact has served as the basis for many tests of renal function, various substances being used, including potassium iodide, methylene blue, fuchsin, lactose, indigo-carmin, and, most widely employed, phenolsulfonephthalein.

The Phenolsulfonephthalein Test. Rowntree and Geraghty, in 1912, introduced the use of phenolsulfonephthalein as a test of renal functional efficiency. This nonirritating dye is promptly and almost completely eliminated in the urine and constitutes at the present time one of the most widely employed measures for the estimation of renal functional capacity.

The bladder having been emptied, the patient drinks 200 to 250 cc. of water. One cubic centimeter of phenolsulfonephthalein solution, containing 6 milligrams of the dye, is injected either intravenously or intramuscularly in the lumbar region with the patient at rest. The injection is made ten to twenty minutes after the ingestion of the water. If the intramuscular route is

of normal clearance. In chronic nephritis, sufficiently advanced to give clearance values 20-30 per cent of the average normal, concentration tests may show minimal urine specific gravity and then reveal no further changes during subsequent progress of the condition, while the clearance continues to fall until, in the terminal stages, it reaches the low level of 3-5 per cent of normal. It appears, therefore, that the fall in urine specific gravity does not show the same uniform relationship to the severity of the renal lesion that is manifested by the degree of diminution in urea clearance.

(3) However, in studying patients with nephritis, concentration tests may be used to advantage to supplement the urea clearance for the following purposes: (a) When a concentration test yields urines of more than 1.026 specific gravity, it may usually be assumed that renal function is normal and the urea clearance test may be omitted. (b) In patients recovering from acute glomerulonephritis, persistent low urinary specific gravities may continue to show evidence of residual renal abnormality for several weeks or months after the urea clearance has returned to normal. The concentration tests are therefore significant in assisting to decide when recovery is complete.

Urea in Other Body Fluids

Because of its extreme diffusibility, urea exists in the lymph, spinal fluid, bile and pancreatic juice in practically the same concentration as in the blood. Considerable interest has been attached to the investigation of the nonprotein nitrogen and urea contents of the perspiration because of the possibility of stimulating its elimination through this channel in patients with renal insufficiency. It has been found that the nonprotein nitrogen content of the perspiration of normal individuals averages about 30 per cent greater than that of the blood, about 65 per cent of the total N.P.N. consisting of urea nitrogen. Whereas under ordinary circumstances the amount of nitrogen lost through the skin is quite small, it may be greatly increased if perspiration is profuse. However, it is doubtful that this channel of elimination can be used to practical advantage in patients with nitrogen retention since the coincident elimination of relatively large amounts of water by the skin may tend to increase rather than to decrease the level of blood nitrogen. The significance of the concentration of the various nonprotein nitrogenous substances in the cerebrospinal fluid has been considered elsewhere (p. 507).

Hensch and Aldrich found that the urea content of saliva, as determined by the urease method, is approximately 80 per cent of that of the blood. The combined urea and ammonia nitrogen

following intravenous injection. An equal amount is eliminated by each kidney in one hour or, if the test is prolonged, in the two-hour period. In the presence of unilateral renal disease the appearance time is delayed on the diseased side and the quantity eliminated is diminished in proportion to the degree of renal functional impairment. It is important to keep in mind the fact that the opposite kidney, if functioning normally, should excrete not only the 50 per cent of the total normally excreted by both kidneys, but also that portion not eliminated by the diseased kidney. In a few instances the ureteral catheters themselves may cause some inhibition of renal function.

If correctly performed, and interpreted with the realization of the possible important part played by extrarenal factors, the phenolsulfonephthalein test constitutes a most valuable test of renal function. In most cases retention of the dye parallels the retention of nitrogen in the blood. The factors which were considered in the discussion of the value of the determination of blood nonprotein nitrogen in renal disease apply equally to the phenolsulfonephthalein test.

Chapman and Halstead recommend the use of the fractional method of estimating the elimination of phenolsulfonephthalein. The test is performed as follows: The bladder is emptied and the patient drinks 600 cc. of water. After thirty minutes, 1 cc. (6 mg.) of phenolsulfonephthalein is injected intravenously. Urine is collected 15, 30, 60 and 120 minutes after the injection. The 15-minute specimen alone is sufficient for routine practical purposes.

Normal excretion values are as follows: 15-minute specimen, 28-51 per cent, average 35 per cent; 30-minute specimen, 13-24 per cent, average 17 per cent; 60-minute specimen, 9-17 per cent, average 12 per cent; 120-minute specimen, 3-10 per cent, average 6 per cent; total two-hour excretion, 63-84 per cent, average 70 per cent. Although flattening of the curve of elimination is of some significance, the elimination of less than 25 per cent of the dye during the first fifteen minutes appears to be the most significant evidence of renal functional impairment yielded by this test. This procedure is of great value in estimating the functional activity of each kidney (ureteral catheterization). As in the case of the ordinary phenolsulfonephthalein excretion test, it is necessary to know that there is no urinary retention and that the bladder has been completely emptied at the end of each period. Abnormal results were obtained by this procedure in about 32 per cent of a series of patients with renal disease in whom normal results were obtained by the ordinary procedure. Some believe that this fractional phenolsulfone-

employed, ten minutes are allowed for absorption of the dye, the bladder being emptied one hour and ten minutes and again two hours and ten minutes following the injection. The specimens are kept in separate containers. Sodium hydroxide (10 per cent solution) is then added to the urine until a maximum red color is produced. Each of the two specimens is diluted to 1000 cc. with water and compared with a standard solution containing 6 mg. of phenolsulfonephthalein in 1000 cc. of water. The comparison may be made in any standard colorimeter. A normal individual eliminates 40 to 60 per cent of the dye in the first hour and 20 to 25 per cent in the second hour, the total two hour elimination being from 60 to 85 per cent. However, all values above 50 per cent in the two hour period must be considered to be within normal limits.

Two important facts must be kept in mind in interpreting results obtained by the phenolsulfonephthalein test. First, extremely low values may be obtained in the presence of factors which tend to cause prerenal deviation of water; second, as is the case with many tests of renal function, a normal quantity of the dye may be eliminated during compensated stages of renal functional impairment in spite of the fact that the renal lesion may be relatively far advanced. It has been demonstrated that whereas in normal individuals the quantity of dye eliminated is independent of the urine volume, in the presence of severe impairment of renal function the output of phenolsulfonephthalein varies more or less directly with the volume of the urine and can be increased by the liberal administration of water. In the absence of extrarenal factors, delayed or incomplete elimination of the dye is indicative of renal insufficiency, the degree of functional injury being roughly reflected quantitatively in the diminution of phenolsulfonephthalein excretion.

Under normal conditions a certain proportion of the injected phenolsulfonephthalein is removed from the blood stream by the liver. In patients with impaired hepatic function this fraction is diminished, the urinary excretion of the dye being correspondingly increased. Consequently, in cases in which hepatic disease is present together with renal disease, the urinary elimination of phenolsulfonephthalein may be greater than if the liver function were normal. Under such circumstances, low normal results may be misleading.

This test may be employed to determine the functional capacity of each kidney. Ureteral catheters are introduced and the test performed as outlined above. In normal individuals the dye appears from both sides usually in five to ten minutes following intramuscular injection, and three to five minutes

contains approximately 13.4 Gm. of nitrogen, 8.5 Gm. of salt, 1760 cc. of fluid and a considerable quantity of purine material in meat, soup, tea and coffee. The dietary is divided into three meals taken at 8 A. M., 12 noon and 5 P. M. No food or fluid of any kind must be taken between meals or during the night.

The bladder is emptied at 8 A. M. immediately before breakfast, the urine specimen being discarded. The urine is collected at two-hour intervals during the twelve-hour day period from 8 A. M. until 8 P. M. The night urine is collected as a single specimen for the twelve-hour period from 8 P. M. until 8 A. M. of the succeeding day.

TABLE 9
TWO-HOUR SPECIFIC GRAVITY TEST (MOSENTHAL)

Time	Normal		Early nephritis		Terminal nephritis		Myocardial failure	
	Cc	Sp. gr.	Cc	Sp. gr.	Cc	Sp. gr.	Cc	Sp. gr.
8 A. M. to 10 A. M . . .	150	1.015	75	1.016	140	1.005	65	1.024
10 A. M. to 12 noon. . .	155	1.020	70	1.017	160	1.004	80	1.022
12 to 2 P. M	190	1.013	100	1.010	150	1.006	70	1.020
2 to 4 P. M	250	1.010	130	1.009	180	1.004	85	1.022
4 to 6 P. M	120	1.020	150	1.010	135	1.005	100	1.018
6 to 8 P. M	245	1.011	180	1.009	115	1.005	50	1.024
Total day	1100	705	..	880	..	450	..
8 P. M. to 8 A. M . . .	360	1.022	700	1.012	970	1.007	150	1.020
Total twenty-four hours	1470	..	1405	..	1850	..	600	..
Ratio day:night	3:1	..	1:1	..	1:1.1	..	3:1	..

The normal individual yields specimens the specific gravity of which varies 10 points or more from the highest to the lowest; the specific gravity of the night urine is 1.018 or more; the total quantity of urine passed during the twelve-hour day period is three to four times as great as that eliminated during the twelve-hour night period. As the renal lesion progresses and the ability of the kidneys to concentrate solid substances in the urine diminishes, the specific gravity becomes fixed at relatively low levels, regardless, within certain limits, of the urine volume. During this stage there may be no manifestations of renal insufficiency, for the kidneys, by increasing the elimination of water, compensate for the diminution in their ability to concentrate solids. Finally, the compensating mechanism is no longer able to meet the demand set by the decreasing ability to concentrate, and renal insufficiency ensues with retention of nonprotein nitrogenous elements in the blood. During the period of renal

phthalein test is quite as informative as the urica clearance test and reflects the diminishing function in progressive renal disease.

CONCENTRATION TEST OF RENAL FUNCTION

Perhaps the first indication of renal functional impairment is the diminution in the ability of the kidney to eliminate the solid constituents of the urine in their normal concentration. Several tests have been proposed for the determination of the concentrating ability of the kidneys. The urica concentration test has been discussed previously. Perhaps the most satisfactory procedures are those which involve the determination of urinary specific gravity under different conditions. The normal kidney possesses the power of eliminating solids regardless, within wide limits, of the amount of water available for their solution. Consequently, urine of low volume has a high specific gravity, that of large volume a low specific gravity. When renal function is impaired, the ability of the kidneys to elaborate a concentrated urine is diminished. This functional aberration is termed hyposthenuria. With increasing renal damage the kidney loses its ability, not only to concentrate, but also to dilute urine. As renal functional impairment progresses the molecular concentration of the urine approaches that of the protein-free blood plasma (isosthenuria). In other words, the maximum possible specific gravity diminishes and the minimum increases, the specific gravity of the urine being relatively fixed within narrowing limits above and below 1.007, the specific gravity of protein-free blood plasma. Diminished ability to concentrate the urine occurs only as a result of renal functional impairment, and is not affected by extrarenal factors. Investigation of the concentrating ability of the kidneys constitutes one of the few measures by which renal functional impairment can be demonstrated during compensated stages. In estimating the urinary specific gravity in specimens containing albumin or glucose, a correction of 0.003 must be subtracted from the observed specific gravity for each gram of albumin and 0.004 for each gram of glucose per 100 cc. of urine. The values stated subsequently in this discussion refer to nonprotein urinary specific gravity.

Two of the most satisfactory procedures employed for the investigation of the concentrating power of the kidney are (1) the two hour specific gravity test and (2) the concentration test.

The Two-hour Specific Gravity Test. This test, originally proposed by Hedinger and Schlayer, and later modified by Mosenthal, consists essentially of the investigation of the urinary response to a diet containing a reasonable amount of fluid, salt and protein. The test meal suggested by Mosenthal

the urine was so severely diminished that they could not, when placed under appropriate conditions, reach a maximum specific gravity of at least 1.010.

The following procedure has been recommended by Lashmet and Newburgh:

(1) At 10 P. M. stop all fluids and food except special diet until the end of the test period (38 hours).

(2) At 8 A. M. the following morning, empty bladder and discard urine. A special diet, containing 40 Gm. protein, 104 Gm. fat, 204 Gm. carbohydrate, 1900 calories, and 1 Gm. added sodium chloride, divided into three meals, is taken on this day. All urine is collected from 8 A. M. until 8 A. M. the following day.

(3) No food or water is taken on this day until the completion of the test. Urine is collected at 10 A. M. and again at 12 noon.

Under these conditions, normal subjects are able to concentrate urine to a specific gravity of 1.029 to 1.032. Impairment of renal function is indicated by decreasing urinary specific gravities below 1.028. Correction should be made in this, as in other concentrations tests, for albumin in the urine. In our experience, this procedure possesses little if any advantage over the simpler concentration test outlined above.

As in the case of the two-hour specific gravity test the urine concentration test aids in differentiating prerenal from extrarenal factors and is capable of indicating renal functional impairment early in the course of renal disease when the functional defect is well compensated. In patients with edema, if the edema fluid is being evacuated, the test period urine may be of low specific gravity, simulating diminished concentrating ability. Obviously, under such conditions a low maximum specific gravity is not necessarily indicative of renal disease. A maximum specific gravity below 1.020 is of serious prognostic import.

This simple test is perhaps the best clinically available test of renal function with the exception of the blood urea clearance test previously described. Practically all information desired regarding the state of renal function may be obtained by the use of the concentration test in conjunction with the estimation of the nonprotein nitrogenous constituents of the blood. The former indicates the presence or absence of renal functional impairment; the latter indicates whether this is in the compensated or uncompensated stage.

The relative values of the concentration tests and the determination of blood urea clearance have been discussed elsewhere (p. 379). The latter usually gives more definite

failure the urine is constantly of low specific gravity whether the urine volume be extremely large or rather small.

Diminution in concentrating ability with a relatively low fixed specific gravity and an increased night urine volume are found in conditions other than primary organic renal disease. Renal functional impairment may exist in association with lesions of the lower urinary passages such as cystitis, pyelitis, ureteral calculi, prostatic disease, etc. Renal function may also be impaired in conditions associated with marked anemia, particularly pernicious anemia. Diabetes insipidus is likewise associated with and characterized by inability of the kidneys to concentrate solids. The two-hour specific gravity test and other tests of the concentrating ability of the kidneys aid in determining the part played by renal and extrarenal factors in the production of manifestations of renal functional insufficiency. For example, in the presence of factors which cause prerenal deviation of water, such as myocardial decompensation, when the concentration of nonprotein nitrogen in the blood may be increased and the elimination of phenolsulfonephthalein may be diminished, the specific gravity of the urine is fixed at relatively high levels. This finding at once excludes the possibility of primary renal insufficiency (renal disease) as the cause of such manifestations.

This procedure, because of its availability and ease of performance, constitutes one of the most satisfactory measures for the estimation of renal functional efficiency. However, it possesses no advantage over the simpler urine concentration test outlined below insofar as the practical demonstration of renal functional impairment is concerned.

The Urine Concentration Test. The test suggested by Addis and modified by Fishberg is as follows:

At 6 o'clock on the evening before the test the patient ingests a meal which should not contain more than 200 cc. of fluid but which has a high protein content. After this no fluid or food is taken until the test period is over. The bladder is emptied before retiring and the urine is discarded as is all urine passed during the night. The bladder is emptied at 8 A. M., 9 A. M. and 10 A. M., each specimen being kept in a separate bottle. The specific gravity of each of the specimens is taken. In normal individuals the specific gravity of at least one of the specimens will exceed 1.025, figures as high as 1.032 being frequently obtained. With increasing renal functional impairment the maximum specific gravity diminishes, approaching 1.007, the specific gravity of protein-free blood plasma. Fishberg states that he has been unable to find any patients with renal disease whose ability to concentrate

kidneys. As is true of nitrogenous waste products, increase in the concentration of inorganic phosphate in the serum does not occur during compensated stages of renal functional impairment and represents a manifestation of renal insufficiency. In most cases the degree of hyperphosphatemia roughly parallels the increase in blood creatinine. It therefore constitutes a valuable index of prognosis in chronic nephritis. Values as high as 24 mg. per 100 cc. have been reported in cases of chronic nephritis, a concentration of 10 mg. per 100 cc. usually indicating an early fatal termination. In young children, during the period of active skeletal development, the normal values for serum inorganic phosphate are 2-3 mg. higher than in the adult.

Deficient elimination of acid phosphate by the kidneys, with its consequent retention in the blood and tissues, is an important factor in the production of acidosis in nephritis. Whereas acidosis may be present in the absence of inorganic phosphate retention, it is almost invariably present when such retention exists. The level of serum phosphate is therefore not an accurate index of the degree of acidosis in patients with nephritis.

Sulfate Retention. Recent studies emphasize the possible importance of sulfate retention in nephritic acidosis. Some observers believe that phosphates and sulfates account for the total increase in undetermined acid in that condition. Others have found a consistent relationship between advanced renal damage and phosphate and sulfate retention in the blood. The normal inorganic sulfur content of blood serum is 0.5 to 1.1 mg. per 100 cc. An increase occurs only in the late stages of nephritis, particularly when there is associated retention of nonprotein nitrogenous elements and inorganic phosphate. Findings above 5 mg. per 100 cc. are extremely rare but have been reported.

Ammonia Formation. Urinary ammonia is derived from blood amino acids, the transformation occurring in the kidneys. Acids can be eliminated by the kidneys only in combination with basic elements, *i.e.*, as salts. If an excessive amount of acid is introduced into or formed in the body, depletion of the base supply of the body would soon occur if ammonia were not provided for the neutralization of the acids in the process of their excretion in the urine. Urinary ammonia is increased in conditions associated with a tendency toward acidosis (except nephritis) and is diminished in conditions of alkalosis (see pp. 275, 282, 285).

The ratio between urinary ammonia and the titratable acid of the urine may be regarded as an expression of the ammonia synthesizing capacity of the kidneys. This ratio has been found to vary normally from 0.7 to 2.8, being usually greater than 1.

information regarding the actual degree of renal functional impairment, but the former is quite as sensitive usually in detecting the presence of such impairment. From a practical standpoint, if normal results are obtained by the concentration test, renal function may be regarded as normal and determination of the blood urea clearance is superfluous. As has been mentioned (p. 379), the concentration test is much more sensitive than the urea clearance test in demonstrating the renal abnormality in essential hypertension and also after partial loss of renal substance by destructive lesions (tuberculosis, malignancy), and partial nephrectomy. According to Fishberg,²² hyposthenuria may be due to (a) reduction in the number of functioning nephrons, (b) damage to the tubular epithelium and (c) decrease in renal blood flow with increase in the relative number of functioning glomeruli or relative increase in the volume of glomerular filtrate.¹⁷

THE ACID-BASE BALANCE IN RENAL DISEASE

The kidneys play an important part in the maintenance of the normal acid-base equilibrium. This subject is discussed in detail in the consideration of the acid-base balance (p. 275). Certain of the factors will be briefly reviewed insofar as they bear upon the determination of renal functional efficiency.

The kidneys help to maintain the normal hydrogen ion concentration of the blood by (1) the excretion of phosphates, (2) the excretion of sulfates, (3) the formation of ammonia and (4) regulation of sodium excretion (p. 275).

Phosphate Excretion. One of the most important functions of the kidney in this connection is its ability to transform basic phosphates (B_2HPO_4) to acid phosphates (BH_2PO_4). It has been demonstrated that in blood plasma (pH 7.4) only approximately 20 per cent of the inorganic phosphate exists in the form of the acid salt (BH_2PO_4); with increasing acidity, as in urine of pH 6, as much as 85 per cent may be in this form; in urine of pH 5 as much as 98 per cent may appear as acid phosphate (BH_2PO_4). In conditions which tend to produce acidosis there is an increase both in the total phosphate excretion and in the proportion of the fraction present in the acid form. A decrease occurs in conditions which tend to produce alkalosis.

The inorganic phosphorus content of blood serum (adult normal 3-4.5 mg. per 100 cc.) is increased in some cases of nephritis with retention of nonprotein nitrogenous substances in the blood. The cause of phosphate retention in nephritis cannot be definitely stated. It is probably not purely the result of the imperfect excretory and concentrating ability of the

kidneys. As is true of nitrogenous waste products, increase in the concentration of inorganic phosphate in the serum does not occur during compensated stages of renal functional impairment and represents a manifestation of renal insufficiency. In most cases the degree of hyperphosphatemia roughly parallels the increase in blood creatinine. It therefore constitutes a valuable index of prognosis in chronic nephritis. Values as high as 24 mg. per 100 cc. have been reported in cases of chronic nephritis, a concentration of 10 mg. per 100 cc. usually indicating an early fatal termination. In young children, during the period of active skeletal development, the normal values for serum inorganic phosphate are 2-3 mg. higher than in the adult.

Deficient elimination of acid phosphate by the kidneys, with its consequent retention in the blood and tissues, is an important factor in the production of acidosis in nephritis. Whereas acidosis may be present in the absence of inorganic phosphate retention, it is almost invariably present when such retention exists. The level of serum phosphate is therefore not an accurate index of the degree of acidosis in patients with nephritis.

Sulfate Retention. Recent studies emphasize the possible importance of sulfate retention in nephritic acidosis. Some observers believe that phosphates and sulfates account for the total increase in undetermined acid in that condition. Others have found a consistent relationship between advanced renal damage and phosphate and sulfate retention in the blood. The normal inorganic sulfur content of blood serum is 0.5 to 1.1 mg. per 100 cc. An increase occurs only in the late stages of nephritis, particularly when there is associated retention of nonprotein nitrogenous elements and inorganic phosphate. Findings above 5 mg. per 100 cc. are extremely rare but have been reported.

Ammonia Formation. Urinary ammonia is derived from blood amino acids, the transformation occurring in the kidneys. Acids can be eliminated by the kidneys only in combination with basic elements, *i.e.*, as salts. If an excessive amount of acid is introduced into or formed in the body, depletion of the base supply of the body would soon occur if ammonia were not provided for the neutralization of the acids in the process of their excretion in the urine. Urinary ammonia is increased in conditions associated with a tendency toward acidosis (except nephritis) and is diminished in conditions of alkalosis (see pp. 275, 282, 285).

The ratio between urinary ammonia and the titratable acid of the urine may be regarded as an expression of the ammonia synthesizing capacity of the kidneys. This ratio has been found to vary normally from 0.7 to 2.8, being usually greater than 1.

In chronic nephritis, particularly during the uncompensated stage, this mechanism is disturbed. The ratio of ammonia to titratable acid in the urine may be extremely low. When renal functional impairment progresses to such a degree as to interfere with the excretion of acids this ratio may be maintained at a constant level or may at times increase. Inefficiency of the ammonia-forming mechanism is an important factor in the etiology of nephritic acidosis. Depletion of the alkali reserve of the blood is the immediate cause of acidosis in nephritis. As has been mentioned previously, this alkali deficit is due in part to the retention of acid phosphate and sulfate in the blood and tissues. However, a considerable fraction of the plasma anion increment in nephritic acidosis consists of radicles the exact nature of which has not been determined. They appear to be probably organic acid radicles and unquestionably contribute largely to the development of acidosis in this condition. The diminution in available basic elements incident to their combination with retained acid radicles is aggravated by the excessive urinary excretion of basic elements required for the neutralization of eliminated acids of exogenous and endogenous origin.

Studies of the factors which influence the acid-base equilibrium in nephritis are of little value from the standpoint of early diagnosis of renal functional impairment. However, in the later stages they may yield information of definite prognostic value. The tests most commonly employed in this connection include the determination of the inorganic phosphate concentration of the blood serum and procedures designed to study the state of the acid-base equilibrium (see p. 285).

In some cases of nephritis, particularly those with uremia, vomiting is a common symptom. If this is excessive, large quantities of hydrochloric acid may be lost through this route. This may in a measure offset the tendency toward acidosis and on rare occasions has resulted in actual alkalosis, the pH of the blood rising to 7.6 in one reported case. However, this does not occur frequently because the organism in nephritis appears to have lost its ability to conserve base, an excessive quantity of which is present in the vomitus. Peters and his co-workers have found that the vomitus in uremia frequently contains but little free hydrochloric acid.

Many other tests have been proposed for the estimation of renal functional efficiency. The ability of the kidneys to prevent the passage of the normal protein constituents of the blood plasma into the urine has been discussed previously (p. 108). The procedures which have been considered above represent,

in the opinion of the authors, the most satisfactory methods now available for the estimation of renal functional capacity. Perhaps the most important and most useful from the standpoints of both early diagnosis of renal functional impairment and estimation of the degree of renal damage is the blood urea clearance test of Van Slyke and his associates. This is in many, but not in all, respects equalled by the concentration test and the two-hour specific gravity test. The phenolsulfonephthalein excretion test, studies of alteration in the acid-base balance, and determination of the concentration of inorganic phosphate in the serum and of the nonprotein nitrogenous constituents of the blood are chiefly of importance in indicating the onset and degree of renal insufficiency. They afford little information of value during the compensated stages of renal functional impairment, with the possible exception of the fractional phenolsulfonephthalein test.

CHEMICAL FINDINGS IN UREMIA¹¹

The term uremia, as employed here, refers to that symptom complex which is associated with retention in the blood of urinary waste products and which is dependent fundamentally upon marked interference with the functional activity of the kidneys and with the consequences of such interference. It does not include so-called prerenal azotemia (nitrogen retention), which may resemble true uremia in several respects but which depends upon factors which may operate in the absence of renal disease or serious renal functional impairment. Nor does it include so-called pseudouremia, more properly designated hypertensive encephalopathy, a symptom complex which may occur in patients with uremia but which should not be regarded as a part of that syndrome.²²

Renal Function. As implied in the definition of the syndrome, uremia is accompanied by evidences of advanced renal functional impairment. Clearance values are extremely low, the urea clearance being usually less than 5 per cent of the average normal. Phenolsulfonephthalein elimination may range from 0 to 10 per cent in two hours and is usually 0 in the fifteen-minute specimen in the fractional method. There is marked elevation of the non-protein nitrogenous constituents of the blood, the total non-protein nitrogen concentration being usually well over 100 mg. per 100 cc., the urea nitrogen over 70 mg. and creatinine above 5 mg. The urine volume may be normal or even increased, but is usually lower than it was during the period of compensation. Regardless of the urine volume, the urinary specific gravity tends to be fixed at a relatively low level, between 1.007 and

1.012 at maximum concentration (nonprotein urinary specific gravity). Higher values for maximum specific gravity may be obtained in patients in whom renal failure is precipitated by the superimposition of some extrarenal factor, such as congestive heart failure or prerenal deviation of water through fluid restriction, vomiting or excessive diarrhea.

Plasma Protein and Albuminuria. If the plasma protein concentration has been previously low it tends to increase, particularly in chronic glomerulonephritis, in which condition the plasma protein concentration may return to normal during this stage. This is due largely perhaps to the associated dehydration and hemoconcentration. It may be dependent in part upon the diminished elimination of albumin in the urine which not infrequently occurs as the chronic active stage of glomerulonephritis progresses into the terminal stage of uremia. This is due presumably to the complete occlusion of increasingly large numbers of glomeruli with consequent diminution in the abnormal filtration surface area which previously, while blood was able to flow through the inflamed glomerular capillaries, allowed the passage of relatively large quantities of albumin into the glomerular filtrate. This diminution in albuminuria is not observed in all cases.

Acid-Base Balance.³⁹ Advanced renal failure results almost invariably in a state of acidosis, which is therefore an almost invariable manifestation of the uremic syndrome. There is an actual base deficit (sodium) and depletion of the alkali reserve (p. 279). This is contributed to by: (a) Failure of the ammonia-forming mechanism in the kidneys with the consequent loss of excessively large quantities of base (Na) in the urine (p. 280); (b) at times, polyuria, especially in chronic glomerulonephritis, with consequent elimination of excessive quantities of fixed base in the urine (p. 280); (c) excessive vomiting frequently plays an important part in this connection, for the vomitus, although usually lacking free hydrochloric acid, usually contains appreciable amounts of sodium chloride (p. 281); (d) excessive diarrhea, with the loss of large amounts of base through this channel (p. 280); (e) retention in the body of excessive quantities of anion, including phosphate, sulfate and undefined organic acids.

In the rare instances of renal failure in which the gastric juice contains approximately normal quantities of free HCl, excessive vomiting may in a measure offset the tendency toward acidosis. On rare occasions under such conditions an actual alkalosis may be observed, as in one reported case in which the pH of the blood rose to 7.6. Such cases are extremely unusual.

Dehydration. Because of the impaired concentrating ability

of the kidney, the excretion of salts in the urine necessitates the elimination of increased quantities of water. This relative polyuria is an important cause of dehydration in chronic nephritis. This condition is also contributed to by excessive vomiting and diarrhea. It is not unusual for edematous patients to become markedly dehydrated during the period of increasing acidosis in the terminal stages of glomerulonephritis. The subsidence of edema is also contributed to by the increasing concentration of plasma protein which frequently is observed under such circumstances.

Plasma Chloride.³³ In the majority of cases the plasma chloride concentration is somewhat diminished. This is perhaps due largely to vomiting, but is also contributed to by deficient intake, polyuria and diarrhea, which cause a steady drain on the chloride and base reserves of the body. The usually coexisting acidosis may play a part in this connection, the accumulation of fixed acids in the blood plasma resulting in a shift of chloride from the blood plasma to the cells and tissues (see chloride shift, pp. 226, 271).

In many instances the coexisting state of dehydration and hemoconcentration may, to a certain extent, mask the state of chloride depletion of the body fluids and the actual concentration of chloride in the plasma may be within normal limits.

Occasionally, hyperchloremia may be observed. This rarely occurs in advanced stages of chronic glomerulonephritis with uremia except under conditions of excessive sodium chloride administration. It may also occur occasionally during periods of elimination of large quantities of edema fluid in such cases. It is observed at times in patients with complete anuria and in some cases of lower urinary tract obstruction. This finding is, however, exceptional in cases presenting the uremic syndrome.

Inorganic Phosphate. An increase in the concentration of inorganic phosphorus in the serum is a common finding in advanced renal failure. Values as high as 40 mg. per 100 cc. have been reported but the common range is from 7 to 15 mg. per 100 cc.⁷ This phosphate retention is believed to contribute to some extent to the acidosis of renal failure. This increase in inorganic phosphorus in the blood is reflected in the body fluids, including the cerebrospinal fluid. Its concentration in most body fluids, including exudates and transudates, is approximately the same as that in the blood serum, whereas the inorganic phosphate content of cerebrospinal fluid is from 30 to 45 per cent of that of the blood serum in the absence of meningeal inflammation.⁷

Calcium. Some degree of hypocalcemia occurs commonly in advanced renal failure.^{7,34} The decrease in serum calcium con-

1.012 at maximum concentration (nonprotein urinary specific gravity). Higher values for maximum specific gravity may be obtained in patients in whom renal failure is precipitated by the superimposition of some extrarenal factor, such as congestive heart failure or prerenal deviation of water through fluid restriction, vomiting or excessive diarrhea.

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Calcium. Some degree of hypocalcemia occurs commonly in advanced renal failure.^{7,34} The decrease in serum calcium con-

centration appears to be dependent on hypoproteinemia or hyperphosphatemia, or both. On the basis of the partition of serum calcium into diffusible and nondiffusible fractions, the latter being in all probability in combination with serum protein, it is readily understood how a significant diminution in serum protein, other things being equal, may be associated with a decrease in the concentration of serum calcium, the diminution in the latter occurring entirely in the non-diffusible fraction. Such findings have been reported repeatedly in nephritis and nephrosis in the absence of any alteration in the serum phosphate concentration. However, in the great majority of cases of advanced renal failure with uremia the plasma protein concentration is within normal limits or is, at least, not significantly decreased; in such cases, hypocalcemia appears to be dependent upon hyperphosphatemia. Under these circumstances the serum calcium concentration bears a roughly reciprocal relation to the serum inorganic phosphorus concentration. The relationship between serum calcium, phosphorus and protein is discussed in detail elsewhere (p. 175).

In the absence of hyperphosphatemia, the calcium content of body fluids, including transudates and cerebrospinal fluid, remains within relatively normal limits, usually 4.5 to 5.5 mg. per 100 cc. This level is maintained even though the total serum calcium concentration may be diminished as a result of diminished serum protein concentration. Such findings indicate that under these circumstances the nondiffusible fraction of serum calcium may be altered independently of the diffusible fraction. However, the hypocalcemia associated with and dependent upon hyperphosphatemia is usually accompanied by a decrease in the calcium concentration of the other body fluids, including cerebrospinal fluid. In such cases, there appears to be a roughly reciprocal relationship between the concentration of calcium and of phosphorus in the cerebrospinal fluid, as in the blood serum, the calcium content decreasing as the phosphate content increases. These findings indicate that the hypocalcemia associated with hyperphosphatemia is dependent, in part at least upon a decrease in the diffusible fraction of serum calcium as has been shown by a biological method, is accompanied by decrease in the ionized fraction of serum calcium. Some observers attribute the motor irritative phenomena of uremia to diminution in the concentration of calcium ion in the cerebrospinal fluid and blood plasma.^{7,34}

In rare instances of uremia in chronic glomerulonephritis serum calcium concentration may be somewhat increased. Although attributed by some to the combined effects of def-

elimination and accelerated mobilization of calcium from the bones as a result of increased hydrogen ion concentration, this phenomenon is difficult to explain on any well-founded basis.

Magnesium and Potassium. Although some observers have reported an increase in the concentration of magnesium in the serum in uremia, normal values are obtained in the great majority of cases.^{30,34} There is some evidence that the administration of magnesium salts to patients with renal functional impairment of advanced degree may significantly increase the level of serum magnesium and may result in manifestations of depression of the central nervous system.

The concentration of potassium in the blood serum may be elevated in advanced renal failure. This may occur even in the presence of marked deficiency in total base, the deficit under such circumstances occurring chiefly in sodium. In many cases the serum potassium concentration is within normal limits.

Plasma Cholesterol. The plasma cholesterol concentration varies considerably in patients with chronic glomerulonephritis and other forms of renal disease.⁸ The acute and chronic active stages of glomerulonephritis are not infrequently accompanied by an increase in the plasma cholesterol concentration. In the terminal stage, however, the development of uremia is not infrequently accompanied by a decrease in plasma cholesterol which may fall to exceedingly low levels (50-60 mg. per 100 cc.). At the same time the total fat, fatty acid and phospholipid concentrations in the plasma may be increased. A similar lowering of the plasma cholesterol concentration may occur in uremia due to other causes. It appears that in chronic glomerulonephritis a decrease in plasma cholesterol from a previously elevated or normal level is of serious prognostic significance, especially if associated with increasing nitrogen retention.

Many believe that the hypocholesterolemia of uremia is probably due in large measure to contributing factors such as anemia, starvation and cachexia. As is well known, these factors may in themselves produce, or at least be accompanied by a fall in plasma cholesterol and, not infrequently, a rise in fatty acid and phospholipid, constituting a blood lipid picture closely resembling that encountered in uremia. It has been found, however, that hypocholesterolemia of severe degree occurs frequently in terminal states unassociated with nitrogen retention or renal disease, in many of which anemia, cachexia and starvation can be excluded from consideration as important etiologic factors. Among these are coronary artery occlusion, congestive and left ventricular heart failure, peritonitis, pneumonia and certain cases of urinary obstruction due to prostatic

enlargement. There is some evidence that excessive withdrawal of cholesterol from the blood as a result of abnormal stimulation of the activity of the reticuloendothelial system may be of importance in the pathogenesis of this phenomenon.

Carbohydrate Metabolism. The fasting blood sugar concentration is frequently elevated and the glucose tolerance diminished in patients with renal failure. The exact cause of these phenomena has not been determined. Some attribute them to excessive hepatic glycogenolysis dependent upon acidosis, others to poor carbohydrate utilization, while still others believe that they depend upon impaired glycogen formation. They occur more commonly in association with glomerulonephritis and nephrosclerosis than in uremia due to other causes.

Phenol and Other Organic Substances. It has been shown that uremia is usually accompanied by increased concentrations of phenols and related substances in the body fluids.⁴³ The interpretation of this observation is rendered difficult by the nonspecificity of the methods employed for the determination of phenols. As stated by Harrison and Mason, phenol, paracresol, indol and other related substances are formed in the body as a result of processes involving deamination, decarboxylation and oxidation of the aromatic amino acids, tyrosine, phenylalanine and tryptophane. These phenols appear to be formed chiefly, if not entirely, as a result of bacterial action on protein derivatives in the intestines. Under normal conditions, these substances are absorbed from the gastro-intestinal tract and are detoxified by conjugation with sulfuric or glycuronic acids. The adequacy of this detoxification depends upon the functional integrity of the liver, intestines and other organs that may be involved in these processes. This subject has been studied extensively by Becher, who believes that certain of the manifestations of the uremic syndrome are dependent upon the accumulation in the blood and other body fluids of phenols and other aromatic compounds. Some observers attribute considerable significance to high values for indican in the blood serum as an indication of approaching uremia.

Guanidine. As is the case with phenol, the evaluation of the significance of guanidine studies is complicated by the lack of specificity of the methods employed for the determination of this substance. However, several investigators have reported an increase in the "guanidine" content of the blood in patients with uremia and have attributed to this increase some of the toxic manifestations of the uremic state.³⁴

Although there is little evidence that urea retention plays a significant rôle in the development of toxic manifestations, it has

been suggested that, according to the law of mass action, the retention of excretory products in the blood and body fluids may diminish the rate of disappearance of intermediary products which may be toxic and may thereby result in the accumulation of these substances which normally occur only in traces. Mason found that high blood urea concentrations appeared to diminish the rate of disappearance of injected guanidine from the blood. It appears possible, therefore, that urea retention, while of itself relatively innocuous, may be indirectly toxic by favoring the accumulation in the body of poisonous products of intermediary metabolism.

DIAZO TEST IN NEPHRITIS

Andrewes found, in performing the indirect van den Bergh test on the serum of patients with nephritis, that instead of obtaining a pink color (bilirubin) as in normal individuals, an orange-buff color developed which deepened in twenty-four hours. When a strong alkali is added to the serum of normal individuals which has been treated in the above manner, a green color appears; it was observed that in certain patients with advanced nephritis a cherry-pink color developed which lasted a few minutes and then faded, its duration depending upon the intensity of the color change. Andrewes noted that in some cases which gave the orange-buff color reaction the pink color did not develop upon the addition of alkali, perhaps because the substance responsible for the color change was present in too low concentration. Hewitt has found that by boiling the mixture of serum and diazo reagent for thirty seconds the alkali may be added without waiting for twenty-four hours as was necessary in the technic originally proposed by Andrewes.

The nature of the substance responsible for this reaction has not been determined with any degree of certainty. Hewitt has found that alcoholic extracts of normal urine give the same reaction and some observers believe that the responsible substance is an indoxyl compound, probably indican, or possibly, in part, indoxylglycuronate. It is apparently not the cause of uremia, is not toxic, and appears to be formed normally in the intermediary metabolism of the body but is not normally present in the blood serum in sufficient concentration to be readily detectable. The test may be performed as follows:

To 1 cc. of blood plasma or serum add 2 cc. of 95 per cent alcohol. To 1 cc. of the supernatant fluid obtained following centrifugation add 0.25 cc. of the van den Bergh reagent and boil gently for thirty seconds. In the serum of normal individuals there is either no color change or the development of a faint pink

color (bilirubin). A positive uremic reaction consists in the appearance of an orange-buff color. Add a few drops of 40 per cent NaOH. In the case of normal individuals (nonuremic) a green color appears; a positive uremic reaction consists in the appearance of a definite pink or cherry-red color which lasts from a few seconds to several hours depending upon the concentration of the responsible agent.

In the great majority of instances the positive reaction is only obtained in the presence of advanced renal insufficiency as evidenced by marked retention of nonprotein nitrogenous elements, acidosis, and a phenolsulfonephthalein elimination below 10 per cent. On the other hand, cases have been occasionally reported in which a positive reaction is obtained before significant nitrogen retention has occurred. In no case, however, has a positive reaction been obtained in the absence of some evidence of renal insufficiency. This test may offer a rapid means of differentiating between coma due to uremia and that dependent upon other causes. A positive reaction is of value chiefly from the standpoint of prognosis, usually indicating a rapidly fatal outcome. This test obviously cannot be applied in the presence of hyperbilirubinemia which causes the development of a green or violet color following the addition of NaOH which will obscure the light cherry-red uremic reaction.

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Chapter XVIII

Nephrosis

NEPHROSIS is a term applied to a group of disorders characterized anatomically by degenerative lesions of the renal parenchyma. The various groups of disorders commonly included under this designation have been considered in the discussion of albuminuria (p. 116). The form of chronic nephrosis known as lipid nephrosis is of particular interest from the standpoint of its metabolic manifestations. There is considerable controversy as to whether this condition is a distinct entity or whether it merely represents a stage in the development of chronic nephritis. Following the original introduction of the term "nephrosis" by Müller in 1905, Volhard and Fahr, in 1914, placed under this designation a group of cases characterized by oliguria, marked albuminuria, extensive edema, lipoidemia and absence of hematuria, hypertension, cardiac hypertrophy and manifestations of renal insufficiency. Histologically, the kidneys showed tubular degeneration with lipid deposits in the cells of the tubules and in the interstitial tissue, the glomeruli being normal. Munk added to this picture the presence of doubly refractive lipid bodies in the urine. Epstein, in 1917, emphasized the fact that in this condition the total protein of the blood plasma or serum is markedly diminished, the reduction occurring practically entirely in the albumin fraction, with consequent diminution in the ratio of albumin to globulin. He proposed the hypothesis that lipid nephrosis is of extrarenal origin, being primarily a disorder of protein metabolism accompanied by a subnormal metabolic rate. Epstein later suggested that the term "nephrosis" should be discarded as misleading and the condition designated by the more descriptive term "diabetes albuminuricus."

Those who disclaim the existence of lipid nephrosis as a pathologic entity believe that it is merely a form of chronic nephritis with extensive degenerative changes in the tubules; early in the course of the disease the glomeruli show little or no change but later lesions appear which progress into typical chronic glomerulonephritis. The renal lesions result in albuminuria which, if prolonged and excessive, is followed by a

reduction in the concentration of albumin in the blood plasma. The elimination of albumin is the result either of injury to the glomerular and capsular epithelium (Elwyn) or of functional alteration in the glomerular capillary walls which permits the passage of albumin (Christian). Those who affirm the clinical and pathologic identity of lipoid nephrosis believe that glomerulonephritis, when it occurs in association with that condition, is a result of the excessive strain incident to the continued excretion of large quantities of albumin by the glomeruli.

There is little doubt that many cases are designated lipoid nephrosis which are in reality chronic nephritis. Most recent evidence is against the view that the former condition exists as a clinical entity. This controversy need not concern us here. In the subsequent discussion the term "chronic nephrosis" is employed to designate a condition characterized clinically by absence of definite evidence of glomerular involvement and renal functional impairment and morphologically by extensive degenerative changes in the renal tubular epithelium, with no significant change in the glomeruli. Lipoid nephrosis, if a clinical entity, would be the prototype of the group of chronic nephroses. The term "nephrotic syndrome" is usually employed to designate a similar group of clinical and metabolic changes occurring in patients who may or may not present evidence of glomerular damage. The latter designation is perhaps to be preferred until more definite evidence is available as to the existence or nonexistence of chronic nephrosis as an entity apart from chronic glomerulonephritis.

THE BLOOD IN CHRONIC NEPHROSIS (NEPHROTIC SYNDROME)

Certain alterations in the chemical composition of the blood are frequently erroneously considered to be pathognomonic of chronic nephrosis. While certain changes occur characteristically in that disorder, identical findings may be obtained in any condition associated with the loss of large quantities of albumin from the body and may be produced by the prolonged administration of diets containing very small amounts of protein. The diagnosis of nephrosis cannot be made on the basis of alterations in the chemical composition of the blood. These must be considered in conjunction with the history, clinical findings and urinary characteristics.

Plasma Proteins. A constant feature of chronic nephrosis is a marked diminution in the concentration of plasma proteins which may, in some cases, fall as low as 3 Gm. per 100 cc. or less

(sec Plasma Proteins, p. 90). This is usually due almost entirely to a decrease in plasma albumin, which may fall from its normal concentration of 3.6 to 5.6 Gm. per 100 cc. to 2.0 Gm. or less. There is still considerable discussion regarding the mechanism responsible for this marked decrease in the albumin content of the blood plasma.⁸ Those who believe that lipid nephrosis represents one phase of the clinical and pathologic history of chronic nephritis are of the opinion that it is due to the excessive and prolonged albuminuria which occurs as the result of the renal lesion. Others believe that lipid nephrosis is in reality primarily a disturbance of protein metabolism characterized by the excretion in the urine of large quantities of abnormal albumin. As has been stated elsewhere (p. 89), evidence has been produced which indicates that a fraction at least of the serum protein in patients with the nephrotic syndrome differs immunologically and chemically from normal serum protein.^{1,5}

Regardless, however, of whether or not the urinary albumin represents normal plasma albumin, it appears to be rather definitely established that the excessive loss of albumin in the urine is the immediate cause of the decrease in the albumin content of the blood plasma. This is also contributed to, perhaps in large measure, by a diminished capacity on the part of the organism for synthesizing plasma albumin. In some cases, dietary restriction of protein and diminished absorption of amino acids from the intestine as a result of edema of the intestinal mucosa may play an important part in this connection.

A marked decrease in albumin is usually followed or accompanied by an increase in globulin (normal 1.3-3.2 Gm. per 100 cc.), apparently an attempt at compensation for the primary deficiency. As a result of these changes the albumin:globulin ratio (normally 1.5-2.5:1) is decreased, or, in some cases, may be actually reversed. The plasma fibrinogen (normal 200-400 mg. per 100 cc.) may likewise increase, presumably in an attempt to compensate for the diminution in plasma albumin, figures as high as 1000 mg. per 100 cc. having been reported. The relation of the plasma protein content to edema has been discussed elsewhere (p. 89).

Lipemia and Lipodemia. The blood serum in chronic nephrosis may be slightly cloudy or at times almost milky in appearance. This lactescence of the serum is due to an increased lipid content, lipemia and lipodemia being almost constant features of this condition.

The blood cholesterol, fatty acids and phosphatides are included in this increase. In chronic nephrosis, cholesterol may reach exceedingly high levels, figures as high as 1400 mg. per

100 cc. having been reported. One of the striking features of this lipidemia is the increase in cholesterol esters, amounting, in many cases, to from 80 to 90 per cent of the total cholesterol (normal 40-70 per cent). Increase in the fatty acid and phosphatide content of the blood usually parallels the degree of hypercholesterolemia.

It must be remembered that increases in the free and combined cholesterol and in phosphatide and fatty acid content of blood plasma occur in subacute or chronic glomerulonephritis with edema, *i.e.*, with a nephrotic complement, as well as in chronic nephrosis without demonstrable glomerular changes. The mechanism underlying the production of lipidemia in these conditions is not clear. It appears to be consistently associated with the diminution in the serum protein, particularly the albumin fraction, and many regard both phenomena as concomitant manifestations of an underlying metabolic disorder which is not clearly understood. Others are of the opinion that the increase in lipids represents an attempt to maintain the osmotic pressure of the blood plasma which has been diminished by the decrease in protein. As stated by Peters and Van Slyke, nephritic lipidemia must be due either to some defect in the mechanism which ordinarily removes fat from the blood or to some disturbance which causes unusually large amounts of fat to be poured into the blood from the fat depots. It seems probable that the lipidemia of nephrosis is a manifestation of a tendency to mobilize lipids into the blood stream from the body depots, the stimulus to this mobilization perhaps being malnutrition, which is a common feature of this condition.

Nonprotein Nitrogen. The blood nonprotein nitrogen is usually normal in chronic nephrosis. An increase in the concentration of the nonprotein nitrogenous constituents of the blood, occurring during the course of chronic nephrosis, is in most instances indicative of a complicating nephritic lesion. However, high values may occasionally be observed in patients with marked edema and with extremely low urine volume, associated perhaps with a high rate of protein catabolism. Such cases are unusual, but the possibility of nonprotein nitrogenous retention due to prerenal deviation of water must be kept in mind in patients who exhibit rapid response to proper therapeutic procedures. There can be little doubt that nephrosis is associated with a marked disturbance of protein metabolism. Apart from the excessive loss of albumin in the urine, which may amount to 30 Gm. daily, the catabolism of protein is probably also increased. One indication of the depleted state of the body protein is the fact that patients with nephrosis exhibit remarkable

powers of storage of large quantities of nitrogen over long periods when served high calorie diets containing large amounts of protein.

Plasma Chloride. The plasma chloride concentration is usually within normal limits, being occasionally elevated and rarely subnormal. As stated by Peters and his co-workers, the general opinion is that there is no demonstrable relationship between the concentration of plasma chloride and the manifestations of nephrosis. With the growing realization of the fundamental importance of plasma protein deficiency in the etiology of edema there is an increasing tendency to regard chloride retention as due to extrarenal factors. Because of the ready diffusion of sodium chloride between blood and tissues, large quantities of salt may be retained in the body without any appreciable increase in the concentration of plasma chloride. Because of its variability and the difficulty of its interpretation, the determination of this factor in patients with nephrosis is of little practical significance (see plasma chloride, pp. 233, 237).

Serum Calcium. The nondiffusible fraction of serum calcium, normally amounting to 4.5 to 6.0 mg. per 100 cc., is apparently bound in some way to the plasma proteins. In consequence of this nondiffusible combination, proteins increase the solubility of calcium in plasma and serum. Alteration in the plasma protein concentration may, therefore, result in a similar change in the concentration of calcium. In the absence of alteration in serum phosphate, Peters and Eiserson stated that the relationship between calcium and protein in the serum may be expressed by the following formula: $\text{Serum Calcium} = 0.556 \text{ Protein} + 6$. Greenwald has proposed the following formula to express this relationship: $\text{Serum Calcium} = x + 0.875 \text{ Protein}$, in which x is a quantity which varies between 5.0 and 3.7 for different adults and has a value of about 6.3 for infants. The value of x must be determined separately for each analytical series. However, albumin appears to be more directly concerned with this "calcium binding" function of the serum protein than is globulin, and consequently the degree of hypocalcemia does not always parallel the degree of protein reduction. As stated by Schmidt and Greenberg, any number of equations can be derived, all of which will more or less give a thoroughly good representation of any single series, but at the present time a sufficient background of underlying knowledge is not available to allow the derivation of any completely valid equation. Serum calcium values ranging from 5.7 to 9.1 mg. per 100 cc. have been observed in nephrosis. The diminution occurs entirely in the nondiffusible calcium fraction and, since this fraction has

no influence upon nerve and muscle irritability, is not associated with manifestations of tetany.

Acid-base Equilibrium. Uncomplicated chronic nephrosis is associated with no disturbance of the acid-base balance, the bicarbonate content, the CO_2 combining power and the hydrogen ion concentration of the blood plasma being within normal limits. Acidosis develops only with the onset of renal functional impairment dependent upon a complicating nephritic lesion.

THE URINE IN CHRONIC NEPHROSIS

The *urine volume* is extremely variable, corresponding to fluctuation in the edema. The daily urinary output may be 500 cc. or less over periods of several weeks, the urine volume increasing markedly with diminution in the edema. During the oliguric period the urine is deeply colored and of high specific gravity, values over 1.035 being encountered not infrequently. This ability to concentrate solids is striking evidence of the absence of renal functional impairment, urea and other non-protein nitrogenous elements being present in the urine in high concentration. During periods of increasing and maintained edema the chloride content of the urine is very low because of its retention in the tissues. As the edema disappears, chloride is eliminated in large quantities.

Albuminuria is one of the most constant features of chronic nephrosis, being usually greater in degree than that observed in any other condition. As much as 30 Gm. of protein may be lost in the urine in twenty-four hours, some patients losing 20 Gm. daily over periods of several months. Because of the frequency of remissions, the degree of albuminuria varies considerably from time to time. Complete disappearance of urinary protein is, however, rare. Albumin constitutes by far the greater proportion of the urinary protein in chronic nephrosis, the albumin:globulin ratio being usually 10 or more to 1. This excessive loss of albumin is perhaps the most important cause of the diminution in the concentration of plasma albumin.

As in nephritis, *glucose* may be present in the urine in small amounts. In 50 per cent of a group of patients studied by Hawkins, the urine contained more than 0.3 per cent of fermentable sugars during fasting, and more than 1 per cent after glucose ingestion. Normal respiratory quotients and blood sugar findings in such patients indicate that the glycosuria is probably of renal origin.

Cholesterol and other lipids are commonly present in large quantities in the urine of nephrotic individuals. This phenomenon is probably due largely to the increased permeability

of the glomerular filter. Some observers are of the opinion, however, that most of the urinary lipids are derived from the renal tubular epithelium which has undergone lipoidal degeneration. Deposits of cholesterol may at times be observed macroscopically and lipid deposits may usually be seen microscopically in the cells of the renal tubules. Others believe that these deposits are not a result of the degenerative process but rather represent infiltration of the tubular epithelium by lipids reabsorbed from the urine. Munk was the first to describe the doubly refractile lipids, the presence of which in the urinary sediment is rather characteristic of nephrotic lesions. These bodies may be seen in the urinary sediment when examined under a polarizing microscope. Frank hematuria does not occur in pure chronic nephrosis although red blood cells and leukocytes may be present in small numbers in about 50 per cent of cases. Hyaline, granular and fatty casts have been observed in most instances.

RENAL FUNCTION IN CHRONIC NEPHROSIS

Renal function is unimpaired in chronic nephrosis in the absence of a complicating nephritic lesion. The urinary specific gravity is high, the concentration test yields normal results, the blood nonprotein nitrogen is within normal limits and the phenolsulfonephthalein excretion is normal in most cases. In children with the nephrotic syndrome, the urea clearance is frequently higher than the normal calculated value (up to 200 per cent of average normal), as are the inulin and diodrast clearance. The increase in urea clearance is probably due chiefly to an increase in renal blood flow and is influenced considerably by a high protein intake. Occasionally, nitrogen retention and deficient dye elimination may occur in the presence of advanced oliguria due to prerenal deviation of water. Particularly in children, the condition may persist for years without evidence of renal functional impairment. Usually, however, the condition is eventually complicated by a superimposed nephritic lesion which is soon followed by evidence of renal functional damage. From the standpoint of studies of renal function the condition then becomes identical with that considered in the discussion of chronic nephritis.

BASAL METABOLISM IN CHRONIC NEPHROSIS

The basal metabolic rate is frequently subnormal in this condition. Values ranging from minus 20 to minus 35 per cent are not uncommonly observed. Epstein believes that patients with lipid nephrosis exhibit a remarkable tolerance to thyroid extract and has reported marked improvement following the

administration of thyroid extract or thyroxin in large doses. The degree of diminution in the basal metabolic rate appears to bear some relation to the degree of hypercholesterolemia.

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Chapter XIX

Hepatic Function

THE determination of the functional efficiency of the liver by chemical methods, or in fact by any laboratory procedure, offers unusual difficulties for several reasons. One of these is the fact that whereas the multiplicity of duties performed by the liver may in certain cases be equally involved in the presence of hepatic disease, it is probable that in some cases one or more of these functions may be disturbed relatively more than the others. Then, too, in several aspects of its functional activity the liver is so intimately associated with other organs that, in investigating these functions, it is difficult or impossible, as stated by Mann, to delineate definitely the hepatic factor. Moreover, the liver possesses an enormous functional reserve and remarkable regenerative powers. It has been demonstrated that within a few weeks after the removal of an entire lobe the liver may be as large as before the operation. Furthermore, by means of consecutive operations and by the institution of measures designed to inhibit this regenerative tendency, the total hepatic tissue may be permanently reduced to as low as 15 per cent of the normal amount without remarkable impairment of hepatic functional efficiency if a proper dietary regime is maintained. Under such circumstances, however, the liver is not able to withstand untoward conditions as well as in its normal state. This experimentally demonstrated fact may explain the frequent failure to demonstrate functional impairment even in the presence of advanced anatomic changes which may be either primary in the liver or secondary to lesions in the extrahepatic biliary passages. This is particularly true of chronic conditions such as cirrhosis, syphilis, malignancy, and the like, which, because of their relatively slowly progressive nature, are associated with concomitant compensatory regeneration of hepatic tissue with little or no impairment of hepatic functional efficiency. In acute widespread hepatic disease, such as occurs in acute yellow atrophy of the liver, arsphenamine hepatitis, and phosphorus and chloroform poisoning, the investigation of liver function yields more satisfactory results.

In spite of the frequently apparently discouraging results, valuable information may at times be obtained in chronic disease

of the liver and biliary passages by the utilization of certain well established methods of investigating liver function. The routine employment of such procedures is of particular importance as a part of the preoperative study of patients with biliary tract disease, for by the institution of measures designed to increase hepatic functional efficiency, much can be done to diminish postoperative morbidity and mortality in these conditions. The fact is too frequently overlooked that functional tests are designed to indicate the state of functional activity of an organ and not necessarily the presence or extent of morphologic changes in that organ. Obviously, it is possible that an organ such as the liver may be the seat of extensive and even grossly evident chronic disease, and still may be capable of carrying on its functions in an essentially normal manner; conversely, it is also conceivable that the functional activity of an organ may be impaired in the absence of demonstrable morphologic changes. If these facts are kept in mind, results obtained by the application of tests of liver function will have considerable clinical significance. On the other hand, any attempt to interpret functional findings in terms of disease diagnosis or of the extent of hepatic parenchymal change may lead to serious error.

CARBOHYDRATE METABOLISM^{11,19,20,29}

The important part played by the liver in normal carbohydrate metabolism has been considered elsewhere in detail (see p. 2). Glucose, preformed or resulting from intestinal digestion of carbohydrates, reaches the liver through the portal circulation. The portion which is not utilized immediately for the maintenance of body activities is stored chiefly in the liver, as glycogen which, when the necessity arises, is converted to glucose which passes in the general circulation to the tissues. Liver glycogen is also contributed to from two other sources: (1) glucose resulting from gluconeogenesis from protein and perhaps fat, a process which probably takes place in the liver and appears to be influenced by hormones of the anterior hypophysis and adrenal cortex; (2) lactic acid, resulting from breakdown of glycogen in the muscles, passing in the blood to the liver, where it undergoes retransformation into glycogen under normal conditions of hepatic function and insulin action. It has been demonstrated, largely through the brilliant researches of Mann, that the deposit of glycogen in the liver constitutes the reserve for the maintenance of the normal glucose concentration of the blood. This function of hepatic glycogen is not shared by glycogen deposits elsewhere in the body, for, following experimental extirpation of the liver, hypoglycemia

occurs which rapidly progresses to a fatal termination although relatively large quantities of glycogen may be present at the time in the muscles.

The influence of the liver in carbohydrate metabolism depends upon the functional activity of the hepatic cells and is not affected by obstruction to the flow of bile per se. Disturbance of hepatic cell function, as indicated below, may result in (1) a tendency toward fasting hypoglycemia, (2) diminished glucose, levulose and galactose tolerance, (3) diminished blood sugar response to epinephrine, (4) glycosuria and (5) increased blood lactic acid concentration and diminished lactic acid tolerance. The presence of such abnormalities in a patient with jaundice suggests the existence of impaired liver cell function, presumably due to hepatic cell damage, while their absence suggests that hepatic function is normal (uncomplicated obstructive or hemolytic jaundice). These procedures have therefore been employed for the purpose of distinguishing between hepatocellular and obstructive types of jaundice. However, clinical obstructive jaundice, particularly if due to cholelithiasis, is usually accompanied, sooner or later, by a variable degree of hepatocellular damage. Moreover, hyperglycemia, diminished glucose tolerance and glycosuria may be contributed to by associated pancreatic disease, and the possibility of other, unrelated disturbances of carbohydrate metabolism must be taken into consideration. Because of these facts, the value of studies of carbohydrate metabolism in differentiating obstructive from hepatocellular jaundice is distinctly limited, and the results must be interpreted in the light of other findings. This is less true of studies of galactose and lactic acid metabolism than of glucose metabolism.

Fasting Blood Sugar Level. Because of the extensive functional reserve and enormous regenerative capacity of the liver the fasting blood sugar is usually found to be within normal limits in hepatic disease, even though the hepatic glycogen content may be markedly depleted. This is particularly true of chronic disorders such as portal cirrhosis, biliary cirrhosis, syphilis, carcinoma, etc. Values of 50 to 60 mg. per 100 cc. may at times be observed in the terminal stages of cirrhotic processes, particularly in obstructive biliary cirrhosis but occasionally also in portal cirrhosis. Serious and occasionally fatal hypoglycemia may occur after operation for biliary tract disease under general anesthesia which may exhaust the glycogen reserve already diminished by associated but not readily detectable hepatic disease (hepatitis). Hypoglycemia is more commonly observed in acute, extensive forms of hepatic disease.

Values ranging from 25 to 60 mg. per 100 cc. have been reported in cases of phosphorus, cinchophen, chloroform and carbon tetrachloride poisoning and in arsphenamine hepatitis. Acute yellow atrophy of the liver (acute diffuse necrosis) and yellow fever are at times associated with extremely low values (15-50 mg.) which may be an important factor in the fatal termination of such cases. It must be realized, however, that the absence of hypoglycemia, even in these acute disorders, is by no means indicative of the absence of hepatic functional impairment. When one realized the fact that 80 per cent of the total substance of the liver may be removed without producing hypoglycemia, one can appreciate readily why this occurs in extensive hepatic disease only as a terminal event.

The phenomenon of spontaneous hypoglycemia, occurring at times paroxysmally, has also been observed in patients with extreme fatty liver, extensive hepatic cirrhosis and widespread involvement of the liver with carcinoma. The occasional occurrence of temporary periods of hypoglycemia in apparently normal individuals subjected to extremely strenuous exercise, such as marathon running, is due perhaps to temporary exhaustion of the hepatic glycogen reserves. As a result, the balance between the utilization of glucose in the muscles and its liberation into the blood stream from the liver is disturbed, and the blood sugar falls unless glucose is supplied from external sources. Whereas the superimposition of hepatic disease usually further diminishes the already lowered glucose tolerance of diabetics and increases their resistance to insulin, the opposite is observed occasionally. Patients with diabetes have been observed in whom the development of extensive parenchymal damage, as in hemochromatosis, was followed by paroxysmal hypoglycemia.

Cases of biliary tract and hepatic disease are encountered occasionally in which the fasting blood sugar concentration is above normal, returning to normal after improvement in the biliary tract condition. This is explained by some on the basis of a disturbance of the mechanism which regulates glycolysis in the liver in accordance with the utilization of glucose in the tissues and the supply of glucose to the liver (pp. 5, 31.) However, great care must be exercised in such cases in eliminating the possibility of the existence of a state of latent diabetes aggravated by the presence of hepatic disease.

Glucose Tolerance. When the glycogen storing function of the liver becomes seriously impaired, the blood sugar curve following the ingestion of glucose exhibits an abnormally high rise and relatively delayed fall. If the blood sugar concentration rises above the "renal threshold level" glucose will appear in the

urine. In the presence of advanced hepatic disease, particularly in acute conditions, decreased glucose tolerance may frequently be demonstrated. The blood sugar curve usually differs from that characteristically observed in diabetes in that, in contrast to the latter condition, there is a tendency toward fasting hypoglycemia with a relatively rapid rise and fall of the blood sugar level following the ingestion of glucose. If sugar determinations are made upon both arterial and venous blood following the ingestion of glucose it may be found that although there is an excessively high rise in both arterial and venous blood sugar, the arterial-venous difference remains within normal limits or may in some cases be increased. This may prove to be an important point in differentiating between the diminished sugar tolerance of deficient hepatic glycogenesis and that of diabetes, in which condition the arterial-venous difference is characteristically decreased (see Table 1, p. 40). The curve obtained in typical cases of hepatic insufficiency is characterized by:

(a) Normal, elevated or subnormal fasting blood sugar level.

(b) Abnormally high rise in venous blood sugar concentration (above 160 mg.).

(c) The maximum concentration is attained at the end of three-fourths to one and one-half hours, in most instances within one hour.

(d) The blood sugar usually falls rather rapidly and returns to the fasting level within two to three hours. Only in cases of extreme grades of hepatic insufficiency is hyperglycemia of long duration.

(e) The arterial-venous difference is usually normal, or in some instances increased.

(f) Because of the absence of disturbance of utilization of glucose in the tissues, the respiratory quotient and serum phosphate change in an essentially normal manner following the administration of glucose; *i.e.*, the respiratory quotient rises and the serum phosphate falls during the period of increased glucose supply and utilization.

Recent studies suggest that failure of the glycogen storing mechanism of the liver may not be the only factor responsible for the production of diminished glucose tolerance in hepatic disease.¹⁶ The observations of Soskin and his associates led them to conclude that under normal conditions a decreased output of sugar from the liver (decreased hepatic glycogenolysis) is an important although not necessarily the only factor in determining the characteristic fall in the blood sugar curve after the administration of glucose. They believe that in the

presence of hepatic functional impairment there is some interference with the mechanism whereby the liver normally diminishes its supply of glucose to the blood in response to an influx of exogenous sugar. According to this hypothesis, therefore, the deleterious influence of diminished hepatic glycogenesis in contributing to the production of diminished glucose tolerance is supplemented by increased or abnormally prolonged hepatic glycogenolysis, the result being an abnormally high and prolonged elevation of blood sugar following the ingestion of glucose.

Although such findings are commonly obtained in patients with hepatic disease, the determination of glucose tolerance has proven to be of little or no practical value in the study of hepatic function. So many factors are involved in the intermediary metabolism of glucose that the interpretation of minor changes in the blood sugar tolerance curve is extremely difficult. Furthermore, because of the activity of extrahepatic factors in the storage and utilization of glucose, essentially normal findings may be obtained occasionally in the presence of advanced hepatic disease.

A peculiar type of disturbance of glucose tolerance has been described in the condition known as glycogen disease (von Gierke's disease).^{19,37} This is apparently a disturbance of glycogen metabolism which appears in early infancy and is characterized by an abnormal deposit of glycogen in the liver, kidneys, heart and other organs, which become so engorged with glycogen that they assume an enormous size. The peculiarity of this store of glycogen is that it becomes fixed in some way and cannot be mobilized to any extent under ordinary conditions. Because of the extraordinary avidity with which the liver and other tissues remove glucose from the blood and store it as glycogen, together with the abnormal stability of the latter, resulting in diminished glycogenolysis, the fasting blood sugar concentration may be subnormal, values as low as 20 mg. per 100 cc. having been reported. Due to the operation of the same factors, the alimentary blood sugar response exhibits a more gradual and less marked increase than normal and, frequently, a more delayed return to the fasting level. The latter phenomenon is difficult to explain.

Epinephrine Hyperglycemia (see pp. 15, 50). The increase in blood sugar which follows the administration of epinephrine in normal individuals apparently depends upon the presence of adequate amounts of glycogen in the liver. Following the administration of 10 minims of a 1:1000 solution of epinephrine hydrochloride by intramuscular injection, the blood sugar concentration normally increases 35-45 mg. per 100 cc. in three-

fourths to one hour and returns to the resting level in one and three-fourths to two hours. Several observers have noted that in the presence of hepatic disease the degree of rise in the blood sugar concentration is not as great as that observed in normal individuals. In some cases of advanced functional impairment associated with acute hepatic disease practically no rise is obtained. These phenomena are presumably due to the diminution in or depletion of the glycogen reserve to the liver. This test usually yields but little information of value in the early diagnosis of liver disease. Variable results are obtained in chronic liver disease, particularly in portal cirrhosis, although good results have been reported in cases of obstructive biliary cirrhosis. Decrease in or absence of the normal response to epinephrine may frequently be observed in patients with acute diffuse hepatic lesions, such as toxic hepatitis, phosphorus and arsenic poisoning, acute and subacute yellow atrophy of the liver, etc. A diminished blood sugar response to epinephrine has been described also in patients with glycogen disease (von Gierke's disease).

Levulose (Fructose) Tolerance. On the basis of the observation that following hepatectomy in frogs the metabolism of levulose may be disturbed whereas that of glucose is relatively unaffected, Strauss, in 1901, introduced the levulose tolerance test as a method of estimating liver function. The procedure as originally described consists in the administration of 100 Gm. of levulose by mouth and the subsequent examination of the urine for levulose over a period of six hours following the ingestion of the sugar. Strauss believed that about 80 per cent of patients with liver disease exhibit a diminished tolerance for levulose as evidenced by the appearance of this substance in the urine under these conditions. About 10 per cent of normal individuals react positively to this levulose test. At the present time this procedure is believed to be of little practical value. Negative results are obtained in many patients with hepatic disease. Moreover, the renal threshold for levulose is apparently much lower than that for glucose, ranging from 115-130 mg. per 100 cc. of blood and varying within wide limits in normal individuals, thus rendering the interpretation of positive results extremely difficult. This procedure has been largely supplanted by methods which include the determination of the blood sugar concentration at intervals following the administration of levulose.

Levulose Tolerance Test. A sample of blood is withdrawn in the fasting state. 45 Gm. of glucose-free levulose are given by mouth in 200 cc. of water or lemonade. Sugar determinations are made

upon samples of blood withdrawn at one-half hour intervals for two hours. In normal individuals the blood sugar rises 15 mg. per 100 cc. or less and rarely exceeds 130 mg. per 100 cc. Hepatic functional impairment is evidenced by the following phenomena:

(a) A rise in blood sugar of more than 35 mg. per 100 cc. At least one blood sugar reading above 135 mg.

(b) Failure of the blood sugar curve to return to the resting level in two hours.

Although results obtained by this test appear to be more satisfactory than those obtained by the glucose tolerance test it has proved to be of little practical value in the early diagnosis or quantitative estimation of hepatic functional impairment. Positive results are obtained more frequently in the presence of acute lesions of the hepatic parenchyma than in diseases of the extrahepatic biliary passages. In most instances this test yields no information which cannot be obtained more readily and more satisfactorily by other methods. More satisfactory results have been reported when the procedure is modified so that the blood levulose alone is determined rather than levulose plus glucose.^{23,62} Normally the blood levulose concentration, 0-8 mg. per 100 cc. in the fasting state, increases not more than 15 mg. per 100 cc., usually within the first hour after ingestion of 50 Gm. of levulose, and falls to 0-10 mg. within two hours. Increases of 16-30 mg. with a delayed fall to the resting level have been observed in patients with hepatocellular damage and occasionally also in arteriosclerosis.

Galactose Tolerance. Bauer, in 1906, suggested the use of galactose in testing the efficiency of the glycogen storing mechanism of the liver. The most commonly employed tests of galactose tolerance are concerned merely with the elimination of this substance in the urine.

In the cases of females, 40 Gm., and in males, 30 Gm. of galactose are administered in 250-500 cc. of cold water in the fasting state. Urine is collected hourly for five hours following the ingestion of galactose. Each test sample is examined for reducing substances by the Benedict qualitative reagent; if positive, the quantitative Benedict test is performed. Bauer regards a melituria of less than 3 Gm. as of no practical significance. The elimination of more than 4-5 Gm. in the five hour period is assumed to indicate hepatic functional impairment. Many observers believe, with Bauer, that the galactose test gives positive results in catarrhal jaundice, the various types of hepatitis and hepatic necrosis, being negative in simple obstructive jaundice.^{53,55,66} It has been found by some that circum-

scribed liver affections do not diminish the tolerance for galactose unless infection coexists. Negative results are usually obtained in cirrhosis and passive congestion of the liver. In view of these observations, the galactose tolerance test has been suggested as a means of differentiating hepatic parenchymal disorders such as catarrhal jaundice, toxic jaundice and other forms of hepatitis (hepatocellular jaundice) from obstructive jaundice. It has also been advocated as a means of detecting hepatic parenchymal damage occurring as a complication of obstructive jaundice.

Although several investigators regard the galactose tolerance test as of value in differentiating between extrahepatic obstructive and hepatocellular types of jaundice, our own experience and that of the majority of observers coincide with that of Banks, whose conclusions in this connection may be summarized as follows: (1) Although a positive galactose test is almost the rule in cases of acute hepatocellular jaundice of average severity, a negative response may occur in mild or convalescent cases or in cases of long standing, and does not necessarily militate against the diagnosis. (2) A positive response may be obtained in 30-45 per cent of patients with obstructive jaundice of various types. (3) The galactose tolerance test does not uniformly distinguish between obstructive and hepatocellular types of jaundice, although it may furnish corroborative data valuable in doubtful cases. (4) In both varieties of jaundice a strongly positive test (excretion of 6 Gm. or more) should be regarded seriously and, if excretion is continuously high, is probably indicative of serious hepatic parenchymal injury. The borderline group of positive tests, with excretion of only slightly more than 3 Gm. of reducing substance, should not be regarded as conclusive evidence against the presence of mechanical biliary obstruction. The occurrence of positive results by this test in patients with extrahepatic biliary obstruction is not difficult to understand when one recalls the extensive morphologic changes that may and frequently do occur in the liver cells under such circumstances.

Studies of the blood galactose curve after administration of galactose yield results that may be more useful in this connection. In the absence of hyperthyroidism (p. 52), blood galactose values higher than 40 mg. per 100 cc. thirty to sixty minutes after ingestion of 40 Gm. of galactose are suggestive of hepatic functional impairment. Similar findings have been reported in osteitis deformans (Paget's disease).⁵ The intravenous test may be of greater value (p. 52). 97 per cent of patients with cirrhosis, 81 per cent of those with hepatitis and only 18 per

cent of those with obstructive jaundice of less than six months' duration were found to have a blood galactose concentration greater than 20 mg. per 100 cc. (normal, zero) seventy-five minutes after intravenous injection of 1 cc. of a 50 per cent solution of galactose per kilogram of body weight.⁶ This test yields normal results in patients with uncomplicated hyperthyroidism.

Blood Lactic Acid. A portion of the lactic acid that results from the breakdown of glycogen in the muscles is carried in the blood to the liver, where that fraction of it which escapes immediate combustion is converted into glycogen. Normal values for lactic acid in venous blood range from 6 to 20 mg. per 100 cc. In the presence of hepatic functional impairment, with disturbance in the glycogen-forming mechanism of the liver, it can be readily understood how a diminished capacity on the part of the liver for removing lactic acid from the blood and reconvertng it into glycogen may result in an increase in the concentration of lactic acid in the circulating blood. Such an increase has been reported by several observers, values as high as 46 mg per 100 cc. having been observed in some cases of extensive, diffuse hepatic parenchymal disease such as occurs in advanced cirrhosis, arsphenamine hepatitis and other varieties of acute diffuse necrosis of the liver.^{38,48,54,67} However, normal findings are frequently obtained in all types of hepatic disease, and the fact that increased values usually appear only in the terminal stages of these conditions renders the determination of the lactic acid content of the blood of little value from a practical standpoint in the estimation of the state of hepatic function.

It has been found that in some cases of hepatic disease, intravenously injected lactic acid or sodium lactate disappears from the blood more slowly than in the case of normal subjects and, consequently, the "lactic acid tolerance test" has been suggested as a means of studying the glycogenic function of the liver.^{9,59}

The following procedure is advocated by Soffer: A control sample of blood is collected in fluoride before breakfast, after a fasting period of twelve hours. The subject then receives, intravenously, 75 mg. per kilogram of body weight of sodium-d-lactate, in 10-14 per cent solution. Blood samples are collected in fluoride thirty minutes after the injection and blood lactic acid determinations are made. It has been found that the increase in blood lactic acid above the resting control value ranges from about 15 to 42 mg. per 100 cc., the peak being reached at the end of five minutes. Subsequently, the lactic acid concentration of the blood falls rapidly, the major drop

occurring within twenty-minutes, returning approximately to the control level within thirty minutes. It has been found that in the presence of acute diffuse hepatic parenchymal damage there is a definite delay in the utilization of intravenously injected lactic acid, due presumably to the difficulty of conversion of the available lactate into glycogen by the damaged liver. An elevation of 5 mg. or more above the control blood lactic acid value at the end of thirty minutes is regarded as evidence of liver damage. Preliminary observations suggest that essentially normal findings are obtained in patients with jaundice due to extrahepatic biliary tract obstruction. This procedure may therefore prove to be of value in differentiating between obstructive and hepatocellular types of jaundice.

PROTEIN METABOLISM

The liver plays an important part in normal protein metabolism. Its chief function in this connection, in man, appears to be the deamination of amino acids, urea formation, and the formation of fibrinogen and perhaps other blood proteins.

Amino Acids. Under normal conditions the liver is the only site of deamination of amino acids absorbed from the intestines. However, because of the presence of an enormous factor of safety, 90 per cent or more of this organ must be removed before this function is significantly impaired. Complete hepatectomy results in a steadily progressive increase in the concentration of amino acids in the blood. Because of this large functional reserve, the amino acid content of blood and urine is usually within normal limits in the more common diseases of the liver. Elevated values may however be observed in conditions associated with acute widespread degenerative lesions of the liver. This occurs characteristically in acute yellow atrophy, phosphorus, chloroform and carbon tetrachloride poisoning, arsphenamine hepatitis, cinchophen poisoning and in some cases of eclampsia. In these conditions the amino acid nitrogen content of the blood is usually 10-15 mg. per 100 cc., occasionally rising to as high as 30 mg. In one reported case, values above 200 mg. were observed just before death. The increase in amino acids in degenerative lesions of the liver is probably due partly to inefficient deamination of amino acids reaching the liver through the portal circulation and partly to extensive autolysis of hepatic tissue. The increased concentration of amino acids in the blood is associated with a corresponding increase in the amino acid content of the urine. Under such circumstances, leucine, tyrosine, glycine, arginine, phenylalanine and other amino acids may be present in the urine in large quantities. Normally,

amino acids, with the exception of glycocoll present in the form of hippuric acid, exist in the urine in such small amounts that their isolation is practically impossible. Consequently, the positive identification of leucine and tyrosine crystals in the urine has been assumed to be strongly indicative of the presence of an extensive hepatic degenerative process such as occurs in acute yellow atrophy of the liver.

The phenomenon of tyrosinuria has acquired new significance recently as a result of the observations of Lichtman, employing a more delicate method for the quantitative determination of tyrosine in the urine. This author believes that the abnormal amount of amino acids excreted in the urine of patients with acute atrophy of the liver originates chiefly from autolysed liver tissue and that in some cases tyrosinuria may occur in the absence of apparent disturbance in the metabolism of amino acids. He believes that this factor is of significance in the diagnosis and prognosis of diseases of the liver and bile passages. Continuous, massive tyrosinuria (0.9-2 Gm. daily) was found to occur only in cases of acute yellow atrophy of the liver with a rapid and fulminating course. Transitory, minimal and moderate tyrosinuria was observed in cases of subacute atrophy of the liver, in degenerating neoplasm of the liver, in toxic hepatic degeneration and, rarely, in patients with obstructive jaundice of long standing due to stone. Tyrosinuria was not observed in patients with uncomplicated inflammatory lesions of the bile passages but was noted occasionally in the presence of extra-hepatic autolytic foci, such as degenerating tumors of the lung or extensive sloughs of the skin. During the phase of recovery from degeneration of the liver, tyrosine vanishes from the urine, but with a fresh exacerbation of the hepatocellular lesion tyrosine reappears in the urine. Negative findings may be obtained in the stage of repair, as in nodular cirrhosis, or in the terminal stages of subacute yellow atrophy with a critical reduction in the amount of functioning parenchyma of the liver. Increasing tyrosinuria is assumed to indicate a rapidly progressive degenerative process in the hepatic parenchyma. Although this finding appears to be of distinct prognostic value, the absence of tyrosinuria in such cases does not necessarily warrant an optimistic prognosis. Jankelson has reported the presence of free tyrosine in the blood of about 80 per cent of patients with liver disease. He believes this finding is indicative of hepatic parenchymal disease but that the absence of tyrosine in the blood does not necessarily indicate the absence of such a lesion.

Urea. Urea is formed in the liver from ammonia derived from amino acids during the process of deamination. The work of

Mann and his co-workers indicates that the liver is the only important site of urea formation. The fact that the production of urea in the body ceases following total hepatectomy would naturally lead to the assumption that hepatic insufficiency should be associated with a decrease in the urea content of blood and urine. In general, the statements made with regard to the relationship of hepatic disease to the metabolism of amino acids apply equally to urea. Deamination and urea formation are closely related phenomena; since the quantity of urea formed by the liver appears to be determined by the efficiency of the mechanism of deamination, it naturally follows that diminution in the concentration of urea in the blood and urine occurs only in those conditions in which the blood and urinary amino acid nitrogen is increased.

In the majority of normal individuals the blood urea nitrogen concentration ranges from 12 to 15 mg. per 100 cc. However, values of 9-18 mg. per 100 cc. cannot be considered abnormal. The hepatic lesions which produce subnormal values are those which interfere with the process of deamination, and include, as stated above, such conditions as acute yellow atrophy of the liver, phosphorus, arsphenamine, chloroform and carbon tetrachloride poisoning and, at times, eclampsia. In one very severe case of acute toxic necrosis of the liver the blood urea nitrogen fell to 0 shortly before death. Values below 6 mg. per 100 cc. are quite unusual. In conditions such as cirrhosis, syphilis, malignancy and catarrhal and obstructive jaundice, the blood urea nitrogen is usually within normal limits. Subnormal values occurring in biliary tract disease, particularly in obstructive jaundice, are, in the experience of the authors, of serious prognostic significance, since they usually indicate a superimposed lesion of the hepatic parenchyma. The normal ratio of blood urea nitrogen to amino acid nitrogen is approximately 2 to 1. A decrease in this ratio, indicating a decrease in the deaminizing capacity of the liver, is usually significant of advanced grades of hepatic insufficiency. Under such circumstances the total nonprotein nitrogen content of the blood and urine is not altered. Determination of the nitrogen partition characteristically reveals a decrease in the proportion of nitrogen present in the form of urea and a corresponding increase in amino acid and ammonia nitrogen. Because these changes occur only in far advanced hepatic disease they are of serious prognostic significance but are of no diagnostic value.

Uric Acid. Mann and his co-workers presented evidence which suggests that the liver plays an important part in the destruction of uric acid in the body. This is questionable

Following complete hepatectomy they found that large amounts of uric acid are eliminated in the urine. On this basis they suggest that the determination of the uric acid content of blood and urine may be used as a test of hepatic functional efficiency. Such studies have proved to be of little clinical value. Although a slight increase in blood uric acid may be observed in some patients with biliary tract disease, cirrhosis, and obstructive jaundice, such findings are not common. In fact, low values have been reported in severe cases of acute yellow atrophy and in chloroform and phosphorus poisoning.

Guanidine. Minot and Cutler, noting the similarity between the intoxication of hepatic insufficiency due to carbon tetrachloride poisoning and that of experimental guanidine poisoning, studied the "guanidine" content of the blood of animals poisoned with carbon tetrachloride and of patients with acute liver injury and eclampsia. In poisoned dogs the blood "guanidine" was found to be elevated from the normal level of 0.2 to 0.45 mg. per 100 cc. of blood to levels of 1 to 2.5 mg. The severity of the toxic manifestations appeared to be more or less dependent upon the level of blood "guanidine." In cases of chronic liver disease, including carcinoma, syphilitic hepatitis, alcoholic cirrhosis and obstructive jaundice, no significant deviation from the normal level was observed. However, in patients with acute arsphenamine hepatitis, acute catarrhal jaundice, pre-eclamptic toxemia and eclampsia, the blood "guanidine" was found to be definitely increased, ranging from 0.5 to 1 mg. per 100 cc in most instances. These findings have been confirmed by other investigators. This increase in "guanidine" (imido-urea), which in itself exerts a definitely injurious effect upon the liver, is perhaps due to some aberration of protein metabolism dependent upon hepatic insufficiency.

The significance of these observations is rendered questionable because of the lack of specificity of the methods available for the quantitative determination of "guanidine" in the blood. Because of this fact, the employment of this procedure is of little practical value.

Plasma Protein. (a) *Fibrinogen.* Fibrinogen is apparently formed entirely in the liver. It has been repeatedly demonstrated that following total hepatectomy the plasma fibrinogen falls progressively, the body being apparently unable to regenerate this substance in the absence of the liver. Clinically, remarkable variations may be observed in the fibrinogen content of blood plasma in liver disease. Analysis of the reported observations appears to indicate that mild liver injury is associated with normal (200-400 mg. per 100 cc.) or slightly increased

values. In the presence of severe liver damage the fibrinogen content of the blood plasma may fall to extremely low levels, figures as low as 50 mg. per 100 cc. having been observed in cirrhosis. As a general rule, the determination of the plasma fibrinogen content is of little value in the early diagnosis of hepatic disease, but low values are usually indicative of extensive liver damage.

(b) *Albumin and Globulin.* The view that the liver plays a major rôle in the formation of plasma albumin and globulin as well as fibrinogen has received added support in recent years. The work of Holman suggests that the body contains a reserve of protein-building material which is stored, in part at least, in the liver, and which is probably at least 50 per cent. albumin or albumin-building material. It is suggested that tissue and plasma proteins are in a state of dynamic equilibrium, in which stored material in the liver plays an important part. It has been found, for example, that Eck-fistula dogs have difficulty in producing sufficient plasma protein to maintain its concentration above the edema level on a standard low protein diet, the capacity of the organism to form new plasma protein under such circumstances being in some instances reduced to less than 10 per cent of normal.⁴⁵

A moderate to marked reduction in total serum protein concentration has been observed in advanced stages of chronic hepatic disease, including portal cirrhosis, carcinoma, chronic hepatitis and chronic passive congestion and, at times, in acute disorders such as catarrhal jaundice, other forms of acute and subacute hepatitis and acute and subacute hepatic necrosis (malnutrition and impaired albumin synthesis).^{14,48,57} Total protein values as low as 3 Gm. per 100 cc. have been reported in cirrhosis, in which this factor may contribute to the development of ascites and generalized edema. The diminution in serum protein in hepatic disease occurs chiefly if not entirely in the albumin fraction. In some instances, particularly in acute forms of liver disease, serum albumin may be only moderately reduced and the serum globulin increased. This increase in globulin is observed much more commonly in primary hepatocellular disorders than in obstructive jaundice, and in some cases, especially in cirrhosis, may be so great as to more than counterbalance the albumin deficit, the total serum protein concentration being actually increased. In cirrhosis, the increase has been found to occur usually largely in the euglobulin and pseudoglobulin I fractions (p. 94) and, by electrophoresis, in the gamma-globulin fraction. In other types of hepatocellular dam-

age, as arsenical hepatitis and catarrhal jaundice, the beta- and gamma-globulin fractions have been found to be increased.²²

Biliary obstruction is usually not accompanied by diminution in serum albumin concentration unless the condition is of extremely long duration. The decrease in serum albumin in hepatic disease has been attributed by some chiefly to coexisting malnutrition and by others chiefly to interference with regeneration of serum albumin resulting from impairment of liver function.

(c) *Mercuric Chloride Reaction (Takata-Ara Test).*^{42,43} This test was first proposed by Takata and Ara for the diagnosis of lobar pneumonia. They found that when pleural fluid from patients with this condition was added to a solution of sodium bicarbonate and mercuric chloride a precipitation of mercuric oxide occurred. This precipitation was regarded as due to an increase in the globulin content of the fluid. It was subsequently found that a similar reaction was produced by the blood serum of patients with cirrhosis of the liver and other types of severe and extensive hepatic parenchymal damage, as in acute hepatic necrosis. Positive findings have been observed occasionally in patients with chronic passive congestion of the liver and chronic alcoholism. Negative results are usually obtained in catarrhal jaundice, syphilis of the liver, cholelithiasis, cholecystitis and cholangitis, hepatic malignancy and most cases of obstructive jaundice. A positive reaction is also usually obtained with ascitic fluid from patients with cirrhosis of the liver, while ascitic fluid from patients with other conditions usually gives a negative reaction. Because of the frequency with which positive reactions are obtained in patients with hepatic cirrhosis, this test was widely advocated as a means of diagnosis of this condition. However, numerous studies have corroborated the conclusion reached by Jezler that a positive reaction is dependent upon an increase in plasma globulin and may be obtained in a variety of conditions accompanied by this phenomenon, including multiple myeloma, the nephrotic syndrome and other extrahepatic disorders (pp. 95-97). Although a high percentage of positive results (60-90 per cent) is obtained in cirrhosis of the liver, a positive reaction may be obtained in the presence of any type of severe hepatic parenchymal damage. The same is true of other so-called "globulin reactions" (p. 95), including the Bauer and Weltmann reactions and the formol-gel and CO₂ saturation reactions. As would be expected in view of the rarity of marked change in the blood plasma protein concentration in simple obstructive jaundice of relatively brief duration, a negative

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Takata-Ara reaction is the rule in this condition, although a positive reaction may be obtained occasionally.

(d) *Colloidal Gold Curve*. A paretic type of colloidal gold curve has been obtained with blood serum of a large proportion of patients with portal cirrhosis, acute and subacute hepatitis and hepatic necrosis and hepatic malignancy. This is due apparently to a qualitative or quantitative abnormality of the euglobulin or gamma-globulin fraction.^{21,24}

(e) *Cephalin-cholesterol Flocculation Test*.^{24,25,33,47,51,52} Haager found that an emulsion of cephalin and cholesterol may be precipitated when mixed with serum of patients with hepatocellular damage and not with normal human serum. Flocculation of the cephalin-cholesterol emulsion with human serum appears to be dependent chiefly upon an increase in gamma-globulin²³ and may occur in conditions other than hepatocellular damage in which this abnormality is present (pp. 94, 97). Positive reactions may be obtained with normal human serum after heating to 56° C. for thirty minutes or after standing at icebox temperatures for several months, and also with normal, untreated rabbit serum.

When a commercial preparation of cephalin-cholesterol antigen (Difco Laboratories) is employed, a 2 to 4 plus flocculation in forty-eight hours is regarded as a positive result. Positive reactions are obtained in practically all cases of acute hepatitis and hepatic necrosis, in the majority of cases of cirrhosis and chronic passive congestion of the liver, and in certain cases of hepatic malignancy and biliary obstruction. Subsidence of the acute hepatic lesion has been observed to be accompanied by a decrease in the intensity of the reaction and the degree of flocculation is regarded by some as a reflection of the activity of the parenchymal lesion. Regarded in this manner, it is believed by many to be of prognostic value, a persistently strongly positive reaction in cirrhosis, for example, being of serious prognostic significance.

The cephalin-cholesterol test may also be helpful in differentiating obstructive from hepatocellular jaundice. In a patient with jaundice of brief duration, a negative reaction suggests biliary obstruction and a positive reaction hepatocellular damage as the underlying cause. However, a positive reaction may be obtained in prolonged obstructive jaundice as a result of superimposed hepatocellular damage (p. 465). Our experience indicates that this procedure is of great value in the study of patients with jaundice. It appears to be a satisfactory index of the activity of parenchymal damage in the absence of complicating extrahepatic factors, and thus supplements measures designed

to evaluate the efficiency of residual hepatic function (serum bilirubin concentration, dye excretion, urine urobilinogen, hippuric acid synthesis).

(f) *Plasma Prothrombin*.^{4,12,15,18} Prothrombin, a constituent of the blood plasma essential for normal coagulation of blood, is generally believed to be protein in nature, being associated with the pseudoglobulin fraction of the plasma proteins. It is formed in the liver, an adequate supply of vitamin K being necessary for its formation in normal amounts (p. 327).

The hemorrhagic tendency manifested by many patients with obstructive, as well as by some with hepatocellular, jaundice and bile fistula, is dependent chiefly upon severe prothrombin deficiency.^{23,27,43,56,58} At least two factors are of significance in this connection, being necessary for the maintenance of a normal plasma prothrombin concentration: (a) adequate hepatocellular function and (b) absorption of an adequate amount of vitamin K from the intestine. Inasmuch as bile salts are necessary for proper absorption of vitamin K (p. 327), inadequate absorption, with consequent hypoprothrombinemia, may occur (1) when bile is absent from the intestine due to bile-duct obstruction or external bile fistula and (2) when there is deficient formation and excretion of bile salts by the liver. Hypoprothrombinemia may also occur in hepatic disease due to inability of the liver to utilize vitamin K satisfactorily for the formation of prothrombin (hepatitis, cirrhosis, acute or subacute hepatic necrosis, chronic passive congestion, fatty liver, extensive hepatic malignancy, catarrhal jaundice, etc.). A drop in prothrombin also occurs frequently after operations on the biliary tract.²

With the two-stage method for prothrombin determination,⁵⁶ hypoprothrombinemia has been demonstrated in a large majority of patients with jaundice of either obstructive or hepatocellular origin. The one-stage method,^{44,50} which is simpler and more commonly employed clinically, reveals this deficiency in a smaller proportion of cases.⁷² No direct methods are available for accurate clinical determination of prothrombin, the indirect methods in common use representing assays of the thrombin-forming capacity of the plasma under controlled conditions. There is apparently a wide margin of safety in the prothrombin factor, and the coagulation time as measured by usual methods may remain within normal limits until more than 80 per cent of the prothrombin of the blood is lost. When the concentration of prothrombin in the plasma falls below about 30 per cent of normal, the "prothrombin time" becomes longer than twenty seconds (upper limit of normal); with further reduction of the

prothrombin concentration to less than 20 per cent of normal, the "prothrombin time" becomes markedly prolonged. When the latter is longer than forty seconds, indicating prothrombin concentration in the plasma of less than 10 per cent of normal, the existence of a hemorrhagic tendency may be expected.⁴⁹ Experimentally, plasma prothrombin deficiency has been produced in animals by common-duct ligation, partial hepatectomy, biliary fistula, and the administration of hepatotoxic substances such as chloroform and carbon tetrachloride.

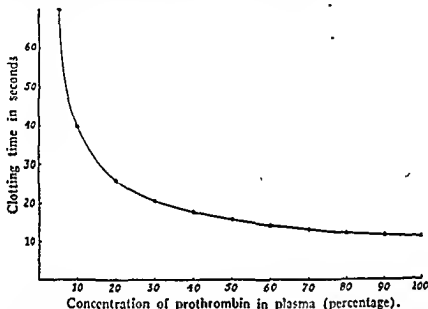


Fig. 15. Relation of clotting time of recalcified plasma (containing an excess of thromboplastin) to concentration of prothrombin. (After Quick.)

Since hypoprothrombinemia in uncomplicated common-duct obstruction (stone, stricture, tumor, pressure) is due to absence of bile salts in the intestine, with consequent inadequate absorption of vitamin K, a prompt increase should follow parenteral administration of the latter or its oral administration in conjunction with bile salts. On the other hand, hypoprothrombinemia in patients with hepatocellular damage is dependent largely upon inability of the liver cells to form prothrombin, and little or no increase should follow administration of vitamin K. These facts have been utilized in attempts to differentiate between obstructive and hepatocellular types of jaundice, the capacity for responding to vitamin K being interpreted as an index of the adequacy of hepatic cell function.^{3,40,41,71} In the presence of hypoprothrombinemia in a patient with jaundice, a significant increase following administration of vitamin K favors the diagnosis of obstructive jaundice, whereas the absence of such increase suggests the presence of impaired hepatocellular function (p. 327). The criteria for a satisfactory response vary

considerably. Some regard a failure to obtain a rise of more than 10 per cent in twenty-four hours after intramuscular injection of a vitamin K preparation (2-methyl-1,4-naphthoquinone) as indicative of hepatocellular damage.⁴⁰ With the oral administration of 8 mg. of this preparation (Menadione) plus 2.5 Gm. of bile salts or, preferably, parenteral injection of 10 mg. of tetrasodium 2-methyl-1,4-naphthoquinone diphosphoric acid ester (Synkovite-Roche), a rise of prothrombin to an essentially normal level may be expected within twenty-four hours in uncomplicated obstructive jaundice and little or no rise in hepatocellular jaundice.³

There is a difference of opinion regarding the value of this procedure as an index of hepatic function and in differentiating between obstructive and hepatocellular jaundice. As stated previously, normal values for plasma prothrombin may be obtained (before giving vitamin K) in a considerable number of patients with obstructive or, more frequently, hepatocellular jaundice, especially when the one-stage method is used. Consequently, normal findings may be misleading. Subnormal values are more significant, but it must be remembered that hypoprothrombinemia may occur in conditions other than diseases of the liver and biliary tract (p. 327). Failure of the plasma prothrombin to respond satisfactorily to vitamin K administration is much more significant of impairment of hepatic function. However, the production of a satisfactory rise does not exclude the possibility of the existence of even considerable hepatocellular damage. The usefulness of this and other procedures in differentiating between obstructive and hepatocellular jaundice is discussed elsewhere (p. 464).

LIPID METABOLISM

The importance of bile in the absorption of fats from the intestine has been recognized for some time, and has been considered in detail elsewhere (p. 132). Its action in this connection may be summarized as follows: (a) activation of pancreatic lipase; (b) the bile salts aid in the emulsification and, consequently, the digestion of fat; (c) the bile salts form soluble addition compounds with fat or fat-soluble substances:

Fat in Feces (p. 135). Obstructive jaundice is usually accompanied by the presence of excessive quantities of fat in the feces (total fat, soap fat, free fatty acids). Similar findings may be obtained in catarrhal jaundice, cirrhosis of the liver, toxic hepatitis and acute yellow atrophy of the liver. These changes have been attributed generally to decreased absorption of the products of fat digestion, a phenomenon which would naturally

be expected to occur when bile is not present in the bowel in adequate amount. However, in view of present knowledge

TABLE 10
PARTITION OF FAT IN FECES (FOWWEATHER)

	Normal* (average)	Obstructive* jaundice
Total fat.....	17.5	50.9
Soap fat.....	4.6	26.8
Free fatty acid..	5.6	18.1
Neutral fat.....	7.3	6.0

* Expressed as percentage of dry matter.

(p. 136) that fecal lipids consist of fatty material secreted or excreted into the bowel as well as unabsorbed portions of ingested lipids, this interpretation is open to serious question. Recent studies indicate that the absence of bile from the intestines causes but little impairment of fat absorption, 65-70 per cent of fatty acids of the diet being absorbed in patients and animals with bile fistula (external). It would appear, therefore, that, as under normal conditions, fecal fat in the absence of bile has, to a large extent, an endogenous origin. Moreover, since the excreted endogenous fat is in the form of neutral fat and fatty acids, it is obviously impossible to interpret alterations in the relative proportions of these substances in the feces in terms of altered digestion and absorption of the ingested fat.

Plasma Cholesterol.^{17,20,23} The alterations that may occur in plasma cholesterol concentration and partition in patients with hepatic or biliary tract disease have been considered in detail elsewhere (pp. 153, 158). The significance of these changes may be summarized as follows:

(a) **Hypercholesterolemia** is found in the majority of patients with uncomplicated extrahepatic obstructive jaundice and occasionally in patients with cholelithiasis without obstruction of the common bile duct. In such cases there is usually little or no significant alteration in the relative proportions of free and ester cholesterol. The increase in plasma cholesterol under such circumstances was formerly attributed to inefficient elimination of cholesterol in the bile. This is now known to be erroneous, the intestinal mucosa being the main site of cholesterol elimination. Moreover, clinical and experimental studies indicate that hypercholesterolemia may occur in cases of external bile fistula, in which this factor of retention is, of course, not operative.²⁰

It would appear from such observations that it is the absence of bile or some of its constituents from the intestines which is the determining factor in the causation of this phenomenon. Release of biliary obstruction is usually followed promptly by a return of the plasma cholesterol concentration to normal levels. Of the utmost importance from a diagnostic and prognostic standpoint is the fact that a similar fall may also occur simultaneously with manifestations of cachexia, terminal cholemia, infection, superimposed hepatocellular damage and other complications, even though the biliary obstruction persists. Under such circumstances, as is indicated below, the fall in plasma cholesterol occurs principally in the ester fraction. Repeated determination of the plasma cholesterol concentration and partition in patients with common duct obstruction is consequently of great value in prognosis and treatment.

(b) Hypercholesterolemia occurs occasionally in patients with mild hepatocellular jaundice (hepatitis), but much less frequently than in obstructive jaundice. When it occurs, it is usually dependent upon an increase in the free cholesterol fraction, the ester cholesterol-free cholesterol ratio being diminished. Except in the terminal stages, plasma cholesterol concentration and partition are usually normal in uncomplicated portal cirrhosis. Hypocholesterolemia is the rule in advanced forms of this condition, the decrease occurring chiefly in the ester fraction, as indicated below.

(c) Conditions accompanied by hepatocellular damage are frequently associated with a diminished proportion of cholesterol esters in the plasma (normally about 60-80 per cent of the total), constituting the so-called "Estersturtz" of Thannhauser and Schaber. This may not be but usually is associated with a diminution in total cholesterol concentration. Among the conditions in which this phenomenon has been observed are acute and subacute hepatic necrosis, phosphorus and chloroform poisoning, arsphenamine hepatitis, toxic hepatitis and yellow fever. In these conditions the plasma cholesterol may be as low as 70-100 mg. per 100 cc. (normal 140-250 mg.), 0-50 per cent being in the ester form (normal 80-200 mg. per 100 cc., constituting 60-80 per cent of the total). In such cases, the degree of hypocholesterolemia and the fall in the ester fraction are often in inverse ratio to the degree of hyperbilirubinemia. It would appear that the degree of diminution in the cholesterol ester fraction in the plasma may be regarded as an indication of the extent of hepatocellular damage in these conditions. This phenomenon also is present when such damage occurs as a complication in patients with extrahepatic obstructive jaundice,

a fall in the ester fraction often occurring in the presence of an initially elevated total cholesterol concentration.

The chief practical significance of this observation lies in the fact that it offers some assistance in differentiating hepatocellular jaundice from simple obstructive jaundice, in which hypercholesterolemia is the rule. Study of the relationship between the degree of bilirubinemia and the changes in plasma cholesterol may be of value in this connection. As stated by Epstein, "in jaundice occurring in acute degeneration of the liver, blood cholesterol does not rise with the bilirubin but usually remains normal or subnormal. This divergence between the degree of blood cholesterol and blood bilirubin elevation in parenchymatous diseases of the liver contrasts sharply with the parallelism between the hyperbilirubinemia and hypercholesterolemia in obstructive jaundice and usually affords a means of differentiating between the two types of jaundice. The cholesterol ester is usually lowered in acute degeneration of the liver and mirrors the severity of the damage." When this phenomenon is observed in patients with common duct obstruction it should be regarded as of serious prognostic significance.

The cause of the fall in cholesterol esters in hepatocellular disease is not definitely known. Various hypotheses have been advanced in explanation of this phenomenon, among which are faulty absorption from the intestine, impaired esterification in the liver and storage of esters in the liver.¹⁰ There is some evidence that bile salts may play an important role in this connection, since it has been known for some time that these substances influence the synthesis and hydrolysis of esters by enzymes (esterases).⁶¹

PIGMENT METABOLISM—JAUNDICE

Bilirubin, the chief pigment of human bile, is derived from hemoglobin by a process of hydrolysis. It has been rather definitely established that the cells of the reticulo-endothelial system, especially those present in the bone marrow, spleen and liver (Kupffer cells), are concerned with the metabolism and formation of bile pigment. Although the hypothesis that the hepatic polygonal cells may play a part in the formation of bilirubin has not been entirely abandoned, it is generally agreed that their chief function in this connection is the removal of bilirubin from the blood stream and its excretion in the bile.⁵² The stages of practical importance in the degradation of hemoglobin are outlined in Fig. 16.

About 25 Gm. of hemoglobin are liberated daily as a result of normal erythrocyte destruction. Approximately 15-20 Gm.

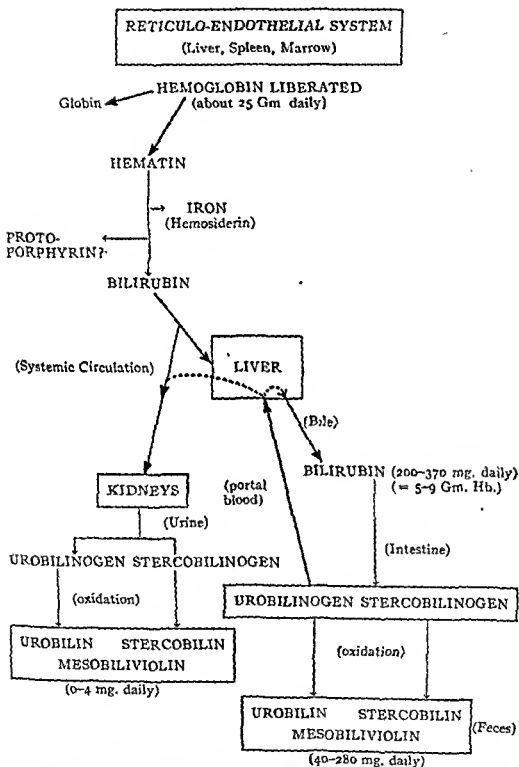


Fig. 16.—Metabolism of hemoglobin. (After Watson, C. J., in Downey's Handbook of Hematology.)

are utilized in the formation of new red blood cells, 5-9 Gm. undergoing degradation to bilirubin. The liver excretes 200-370 mg. of bilirubin daily in the bile, representing about 4 per cent by weight of the hemoglobin from which it is derived. In the upper intestine, this is reduced successively to mesobilirubin, urobilinogen (mesobilirubinogen) and stercobilinogen by the action of putrefying bacteria. A fraction of the urobilinogen is reabsorbed into the portal circulation and is carried to the liver (enterohepatic circulation); the major portion of this fraction is removed from the portal blood by the hepatic cells and is perhaps reconverted into bilirubin or a hypothetical pigment complex, the remainder escaping into the general circulation to be excreted in the urine (0-4 mg. daily). The portion which escapes reabsorption from the intestine is oxidized to urobilin, mesobiliviolin and stercobilin, which are the normal pigments of the feces (40-280 mg. daily, usually 100-250 mg.). There is some question as to whether any bilirubin is absorbed from the intestine; none is present normally in the feces. Inasmuch as the molecular weights of urobilinogen, urobilin, stercobilinogen, stercobilin and mesobiliviolin are approximately the same as that of bilirubin, all being about 4 per cent of that of hemoglobin, the approximate hemoglobin wastage may be determined indirectly by determining the quantity of these pigments excreted in the urine and feces.

Porphyrins are red pigments with a pyrrole structure, which are important components of hemoglobin, myoglobin and other respiratory pigments, such as cytochrome, chlorophyll and catalase. There are four isomeric etioporphyrins, designated Types I, II, III and IV, but these are of practical importance only as reference types, the naturally occurring porphyrins corresponding to Types I and III.^{6,9,14,53} The production of these substances parallels hematopoietic activity and hemoglobin synthesis, Type III being formed in large amounts and utilized for the formation of hemoglobin and other respiratory pigments, which are compounds of Type III porphyrin. Small amounts of coproporphyrin Type I are formed as a by-product in normal hematopoiesis and, perhaps with small amounts of porphyrin Type III not utilized in hemoglobin synthesis, are excreted, largely by the liver in the bile and eventually in the feces and urine, the quantity excreted paralleling that of urobilinogen. Under normal conditions, the daily excretion of coproporphyrin is about 10-120 micrograms in the urine and 100-200 micrograms in the feces, the normal urine: feces ratio being about 0.1 to 0.6.

Clinical investigation of the state of liver and biliary tract

unction with regard to the metabolism of bilirubin and its derivatives includes studies of (a) serum bilirubin, (b) bilirubin tolerance, (c) urine bilirubin, (d) excretion of urobilinogen and related products in the urine and feces and (e) excretion of porphyrin in the urine and feces.

SERUM BILIRUBIN

Qualitative van den Bergh Reaction. The van den Bergh reaction depends upon the fact that bilirubin combines with diazobenzosulfochloride (Ehrlich's diazo reagent) in alcoholic solution to form acetophenolazorubin. This reaction, requiring the addition of alcohol to the serum-reagent mixture, was termed the "indirect" reaction and is a specific test for bilirubin.⁴⁹ The direct mixture of serum and reagent in aqueous-acid solution constitutes the "direct" reaction. The bilirubin of normal serum and that resulting from hydrolysis of hemoglobin ("hemolytic" bilirubin) give only the indirect reaction. The bilirubin of bile gives both indirect and direct reactions. Consequently, the hypothesis has been proposed that some change occurs in the bilirubin molecule or in its mode of combination during its passage through the hepatic cells from the blood to the bile. The production of a positive direct reaction in the blood serum implies the presence of bilirubin that has re-entered the circulation after having been passed out of the blood, through the hepatic cells, into the bile canaliculi (e.g., biliary obstruction or hepatocellular damage) (pp 440ff.).

Certain differences have been described between so-called "direct" and "indirect" bilirubin:² (1) direct bilirubin is more readily oxidized; (2) when the serum proteins are precipitated with alcohol, direct bilirubin is adsorbed on the precipitate more readily than indirect bilirubin; (3) when serum is shaken with chloroform, indirect bilirubin is dissolved and direct is not; (4) direct bilirubin is dialyzable while the indirect is not. The last observation has not received general confirmation.^{7,20} The exact basis for this difference in reaction is not known.^{2,10,27,52} In the past, considerable attention was directed to the possibility that an increase in the concentration of surface-active substances (bile salts, cholesterol, fatty acids) may be responsible for the production of the direct reaction. According to this hypothesis, as bilirubin enters the blood stream after its formation from hemoglobin in the cells of the reticulo-endothelial system, it is adsorbed by some constituent of the blood plasma, probably protein. This adsorption prevents bilirubin from being excreted readily by the kidneys, from being readily oxidized, and from reacting immediately with the diazo reagent in an

aqueous medium, properties which characterize that form of bilirubin which gives only the indirect van den Bergh reaction. On this basis, the direct van den Bergh reaction is obtained whenever substances which lower surface tension are present in the blood stream in excessive amounts. From a pathologic standpoint, bile salts are the most important of these, being present in increased concentration in the serum of most patients with obstructive and many with hepatocellular jaundice, in which conditions the serum characteristically gives a positive direct van den Bergh reaction. However, it has been shown that bile salts are probably not responsible for this phenomenon in clinical jaundice.^{10,32}

Studies employing an improved quantitative method³¹ have shown that although normal serum bilirubin does not give a direct qualitative van den Bergh reaction (no color development within thirty seconds), it contains a fraction which reacts in aqueous-acid solution (direct reaction) in ten to thirty minutes, the magnitude of which varies somewhat in normal serum and in the serum of patients with various types of jaundice (pp. 436, 442).⁸ This demonstration of the presence of a considerable but variable quantity of direct-reacting bilirubin in normal as well as abnormal serum simultaneously with a non-direct-reacting fraction suggests that the essential difference lies either in (a) the bilirubin molecule or (b) its mode of combination in the serum, rather than in other factors in the serum that catalyze or inhibit the direct reaction. There is considerable evidence^{10,32} that the differences in reactivity are dependent on structural rather than catalytic factors, and that all of the bilirubin in human plasma of high bilirubin content is bound to the plasma albumin. However, there is also evidence that the capacity of serum bilirubin for reacting with the van den Bergh reagent in aqueous acid is dependent, in part at least, on factors in the serum other than the bilirubin molecule or the nature of its combination with albumin.⁸

The view that the production of the direct qualitative reaction is dependent primarily upon an increase in concentration of bilirubin in the serum is not tenable. A negative direct reaction has been obtained with the serum of subjects with congenital hemolytic jaundice at bilirubin levels as high as 9-12 mg. per 100 cc. and positive direct reactions in obstructive and hepatocellular jaundice at levels as low as 0.6 mg. per 100 cc. Moreover, there is direct evidence that the liver is implicated in the phenomenon that allows the production of the direct reaction.³³ It is well known that following ligation of the common bile duct the serum bilirubin gives a direct reaction. If

the liver is removed at a time when the direct reaction is given by the serum, the quantity of direct-reacting bilirubin remains unchanged subsequently. However, additional bilirubin accumulates in the blood after hepatectomy, so that the total concentration continues to increase at the same rate as after ligation of the common duct, but the added bilirubin gives only the indirect reaction.

Several types of direct reaction have been described: (a) Immediate direct reaction, in which a red-violet color develops immediately and attains a maximum intensity in ten to thirty seconds, the depth of color being roughly proportional to the amount of bilirubin. (b) Biphasic direct reaction, in which a reddish color appears immediately and changes to a red-violet either rapidly (biphasic prompt) or slowly (biphasic delayed). (c) Delayed direct reaction, in which a reddish color appears in from one minute to several hours, gradually deepening and acquiring a violet hue. It is the consensus at present that any distinction between the immediate and biphasic reactions is of no practical consequence and that the delayed direct reaction has the same significance as a negative direct reaction. The appearance of the characteristic color in aqueous acid within thirty seconds constitutes a positive direct reaction and absence of color development in this time is reported as a negative direct reaction. The clinical significance of these reactions in the presence of hyperbilirubinemia is discussed elsewhere (pp. 440, 442). Suffice it to state here that it is generally assumed that the bilirubin in serum that gives a negative direct reaction has not passed through the hepatic cells, and that if the direct reaction is positive, some bilirubin has been reabsorbed into the blood (directly or via lymphatics) from the bile canaliculi (obstruction to flow of bile or hepatocellular damage).

Serum Bilirubin Concentration. The serum bilirubin concentration is usually determined either by a quantitative procedure based upon the van den Bergh reaction or by the icterus index determination.

Quantitative van den Bergh Procedure. The normal serum bilirubin concentration in adults is 0.1–0.8 mg. per 100 cc., being below 0.5 mg. in about 75 per cent of cases. Some regard 0.25 or 0.4 mg. as the upper limit of normal, but a more satisfactory photoelectric method³¹ has established the accuracy of the former figures. Reported values of 1.0–2.8 mg. per 100 cc. in apparently normal subjects probably represent instances of latent "familial" jaundice.¹¹ The serum bilirubin concentration increases in untrained subjects ascending to high altitudes (anoxemia and excessive blood destruction). A state of physio-

logic hyperbilirubinemia is present during the first several days of life (up to 2 mg. per 100 cc. at birth), increasing to a maximum at about the fourth day (up to 11 mg.) and falling subsequently during the following week (up to 10 mg. at nine days).^{12,14} This is due perhaps to a higher rate of blood destruction than in adults, accompanied probably by some degree of inadequacy of hepatic function in removing bilirubin from the blood stream.

Although the qualitative direct van den Bergh reaction is negative, it is possible to demonstrate that normal serum contains both nondirect-reacting and direct-reacting bilirubin.^{8,11} The direct-reacting bilirubin ranges normally from 0.0 to 0.4 mg. per 100 cc., the proportion which it constitutes of the total decreasing from a peak of 75 per cent to about 40 per cent as the total bilirubin concentration increases from 0.2 to 0.8 mg. per 100 cc.

The chief objection to the quantitative van den Bergh reaction in the past was based on the fact that the precipitation of protein incident to the production of the indirect reaction involved a variable loss of bilirubin due to adsorption of the latter by the protein precipitate. However, with a method now available, employing 50 per cent methyl alcohol,³¹ no precipitation of protein occurs and exact quantitative determinations may be made.

Icterus Index.^{3,33} Determination of the icterus index consists merely in comparing the intensity of the yellow color of the blood serum or plasma with that of a standard 1:10,000 solution of potassium bichromate (icterus index of the standard = 1). The icterus index of normal serum or plasma is 4 to 6, i.e., the yellow color is 4 to 6 times as intense as that of the standard. This is obviously an entirely nonspecific method, but is useful clinically because of its simplicity and because the intensity of the yellow color of the serum is usually dependent upon the bilirubin concentration. However, apart from technical difficulties due to hemolysis and turbidity of the serum, which interfere with the color comparison, difficulties in interpretation may arise from the fact that occasionally substances other than bilirubin may impart a yellow color to the serum. The most important of these are xanthophyll and carotin or carotene. In the presence of carotinemia (p. 316) the icterus index is increased and does not, of course, reflect the bilirubin content of the serum. In our opinion, the quantitative van den Bergh reaction, employing the procedure of Malloy and Evelyn,³¹ is superior to the icterus index determination for the purpose of estimating the serum bilirubin concentration.

HYPERBILIRUBINEMIA

The concentration of bilirubin in the blood plasma depends upon (1) the number and rate of destruction of erythrocytes (hemoglobin liberation), (2) the functional activity of reticulo-endothelial cells (bilirubin formation), (3) hepatic polygonal cell function (removal of bilirubin from the blood), (4) patency of the bile canaliculi and bile ducts (normal flow of bile) and (5) structural integrity of the hepatic cell lining of the bile canaliculi (Barron).²

Serum bilirubin concentrations above 0.8 mg. per 100 cc. or icterus index values above 6 constitute a state of hyperbilirubinemia. In the majority of cases, bilirubin concentrations greater than 0.5 mg. are probably abnormally high. The degree of hyperbilirubinemia necessary for the production of clinical icterus varies in different types of jaundice. It has long been recognized that discoloration of the sclerae and the skin and passage of bile pigment into the urine occur more commonly in obstructive and hepatocellular than in hemolytic jaundice. This has been explained on the basis of the hypothesis that in obstructive jaundice the bilirubin is more readily diffusible (direct-reacting bilirubin) than in hemolytic jaundice (non-direct-reacting bilirubin). It has been found that in obstructive and hepatocellular jaundice, when an icterus index value of 16 or a serum bilirubin concentration of 1.6 mg. per 100 cc. is reached and persists for some days, bilirubin diffuses through the capillaries and appears in the tissues and urine. Greater concentrations are usually necessary in hemolytic jaundice for the production of frank icterus. Some observers believe that at times bilirubin leaves the blood for the tissues at a lower concentration than the threshold for elimination in the urine. Once clinical jaundice and bilirubinuria have occurred, they usually persist for some time after the serum bilirubin has fallen below this "threshold" level. During the period of developing jaundice, therefore, serum bilirubin concentrations between 0.8 and 1.6 mg. per 100 cc and icterus index values between 6 and 15 usually represent a condition of latent jaundice, not detectable by methods other than examination of the blood. At relatively low levels of bilirubinemia, the ratio between the icterus index and serum bilirubin concentration, in milligrams per 100 cc., is roughly 10:1, an icterus index of 6 corresponding to a serum bilirubin of 0.6 mg., and one of 15 to a bilirubin of 1.5 mg. This relationship has been found not to be so consistent at higher levels of bilirubinemia,¹⁷ but this may have been due to inaccuracies inherent in serum bilirubin determinations by methods employed formerly.

Diminution in the amount of bilirubin in the blood may be observed in aplastic anemia, chlorosis and in all "secondary" anemias, particularly those associated with malignancy and with chronic nephritis. A decrease may occur in the elevated serum bilirubin concentration of patients with carcinoma of the head of the pancreas even though complete obstruction to the flow of bile persists. This phenomenon is due perhaps to a decrease in the rate of formation of bilirubin as a result of decrease in the rate of destruction of red blood cells.

TABLE 11
CLASSIFICATION OF JAUNDICE
(After Rich)⁴¹

- I. *Retention Jaundice*. Overproduction of bilirubin and subnormal liver function due to the following causes. Negative direct van den Bergh reaction.
 - A. Anoxemia.
 1. Anemia: pernicious; hemolytic; sickle-cell; paroxysmal hemoglobinuria; drugs (pbeaylhydrazine, sulfonamides, etc.); mismatched transfusion.
 2. Chronic Passive Congestion: cardiac decompensation, especially with pulmonary infarction.
 - B. Febrile Diseases. Associated with anoxemia, resulting from
 1. Anemia: hemolytic septicemia; malaria; blackwater fever.
 2. Pulmonary Consolidation: lobar pneumonia.
 - C. Immaturity of Liver Cells. Icterus neonatorum.
 - D. Undetermined: Hanot's cirrhosis.
- II. *Regurgitation Jaundice*. Reflux of bilirubin from canaliculi into blood because of obstruction to outflow of bile or necrosis of liver cells (disruption of continuity of canaliculi). Positive direct van den Bergh reaction.
 - A. Necrosis of Liver Cells, caused by
 1. Toxic Agents.
 - (a) Chemical: chloroform; carbon tetrachloride, phosphorus; arsphenamine; sulfonamides, etc.
 - (b) Vegetable: mushroom poisoning.
 - (c) Bacterial: yellow fever; Weil's disease; congenital syphilis; "catarrhal jaundice" (?), etc.
 - (d) Undetermined: idiopathic "acute yellow atrophy"; portal cirrhosis.
 2. Severe Degrees of IA and B.
 - B. Obstruction of Bile Ducts, due to
 1. Plugging: calculi, biliary and pancreatic; inflammation; parasites (fasciola, ascaris, etc.); neoplasms.
 2. Stricture: (a) scarring, due to chronic inflammation, injury (e.g., postoperative), syphilis;
 - (b) malformations, as congenital stricture or atresia;
 - (c) neoplasms.
 3. Pressure: (a) inflammatory masses in liver (abscess, gumma, tuberculosis, Hodgkin's disease);
 - (b) parasitic masses, as echinococcus cyst;
 - (c) aneurysms;
 - (d) peritoneal adhesions;
 - (e) neoplasms;
 - (f) lymph nodes.

It should be emphasized that the determination of the serum bilirubin concentration is the only certain means of detecting

conditions of latent jaundice and gives a much more definite idea of the severity of and variations in hyperbilirubinemia than does examination of the urine or feces or the study of skin pigmentation. This study is therefore indicated in all cases in which information is desired regarding the possible presence of hepatic functional impairment, biliary stasis or excessive hemolysis. The frequency of latent or frank jaundice in both calculous and noncalculous cholecystitis as well as in hepatic disease, congestive heart failure and a variety of primarily extrahepatic disease processes emphasizes the importance of the estimation of the degree of bilirubinemia in these conditions. Experience has demonstrated the fallacy of attempting to estimate variations in bilirubinemia in patients with jaundice by observing variations in color of the skin and conjunctivae.

TABLE 12
CLASSIFICATION OF JAUNDICE
(After Rolleston and McNee)⁴²

I. *Hemolytic* Excessive production of bilirubin. Negative direct van den Bergh reaction

Examples: Congenital hemolytic jaundice; icterus neonatorum; erythroblastosis fetalis; familial jaundice; paroxysmal hemoglobinuria; pernicious anemia; splenic anemia; Cooley's anemia; Marchiafava-Micheli syn-

forms; poisoning with snake venoms, arseniuretted hydrogen, toluylenediamine, phenylhydrazine, acetanilid, sulfonamides, dinitrobenzol, aniline, benzol and nitro compounds of phenol, etc.

II. *Hepatocellular*. Injury to liver cells. Decreased removal of bilirubin from the blood, due to increased pressure within or rupture of canaliculi. Positive direct van den Bergh reaction.

Examples: Acute and subacute hepatic necrosis; icterus

caused by poisoning with asphyxiating, carbon compounds, mercury, lead, chloroform, carbon tetrachloride, phosphorus, tetrachlorethane, trinitrotoluene, cinchophen; x-ray over liver.

III. *Obstructive*. Obstruction to flow of bile, usually confined to extrahepatic duct obstruction. Reflux of bilirubin from canaliculi into lymphatics or directly into blood, due to increased pressure within or rupture of canaliculi. Positive direct van den Bergh reaction.

Examples: Calculi, biliary or pancreatic; cholangitis; neoplasm of pancreas, ducts, duodenum or lymph nodes; duodenitis and diverticulum of duodenum; parasites in ducts; stricture of ducts, congenital or acquired; adhesions; pancreatitis; pancreatic cysts; cysts, carcinoma or abscess of the liver; aneurysm of the hepatic artery or renal artery.

The most widely accepted classifications of jaundice, based on pathogenesis, are presented in Tables 11 and 12.

Diminution in the amount of bilirubin in the blood may be observed in aplastic anemia, chlorosis and in all "secondary" anemias, particularly those associated with malignancy and with chronic nephritis. A decrease may occur in the elevated serum bilirubin concentration of patients with carcinoma of the head of the pancreas even though complete obstruction to the flow of bile persists. This phenomenon is due perhaps to a decrease in the rate of formation of bilirubin as a result of decrease in the rate of destruction of red blood cells.

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II. Hepatocellular. Injury to liver cells. Decreased removal of bilirubin from the blood and, if severe damage, escape of bilirubin from canaliculi into blood, due to cell necrosis and disruption of continuity of canaliculi. Direct van den Bergh reaction positive after very early stage.

Examples: Acute and subacute hepatic necrosis; catarrhal jaundice; hepatitis due to any infection, as syphilis, pneumonia, diphtheria, etc.

yellow fever; Weil's disease; congestive heart failure; hyperthyroidism; diabetes mellitus; etc.

1

III. Obstructive. Obstruction to flow of bile, usually confined to extrahepatic duct obstruction. Reflux of bilirubin from canaliculi into lymphatics or directly into blood, due to increased pressure within or rupture of canaliculi. Positive direct van den Bergh reaction.

Examples: Gallstones; strictures of ducts; parasites in ducts; stricture of ducts, congenital or acquired; adhesions; pancreatitis; pancreatic cysts, cysts, carcinoma or abscess of the liver; aneurysm of the hepatic artery or renal artery.

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 - (a) Chemical: chloroform; carbon tetrachloride, phosphorus; arsphenamine; sulfonamides, etc.
 - (b) Vegetable: mushroom poisoning.
 - (c) Bacterial: yellow fever; Weil's disease; congenital syphilis; "catarrhal jaundice" (?), etc.
 - (d) Undetermined: idiopathic "acute yellow atrophy"; portal cirrhosis.
 2. Severe Degrees of IA and B.
 - B. Obstruction of Bile Ducts, due to
 1. Plugging: calculi, biliary and pancreatic; inflammation; parasites (fasciola, ascaris, etc.); neoplasms.
 2. Stricture: (a) scarring, due to chronic inflammation, injury (e.g., postoperative), syphilis;
 - (b) malformations, as congenital stricture or atresia;
 - (c) neoplasms.
 3. Pressure: (a) inflammatory masses in liver (abscess, gumma, tuberculosis, Hodgkin's disease);
 - (b) parasitic masses, as echinococcus cyst;
 - (c) aneurysms;
 - (d) peritoneal adhesions;
 - (e) neoplasms;
 - (f) lymph nodes.

It should be emphasized that the determination of the serum bilirubin concentration is the only certain means of detecting

Both direct- and indirect-reacting bilirubin increase in the blood of patients with moderate and severe jaundice due to extrahepatic biliary obstruction (Table 13, p. 440). If the view can be maintained that bilirubin in the blood which does not give the direct van den Bergh reaction has not passed through the hepatic cells, while that which does give the reaction has been reabsorbed from hepatic cells or from the bile canaliculi, it would appear that in the great majority of cases of obstruction the jaundice is a combination of the "regurgitation" and the "retention" varieties. The qualitative direct van den Bergh reaction may be negative in the first few hours of obstruction and, occasionally, following relief of the obstruction, after the serum bilirubin has fallen to about 1.5 mg. per 100 cc. The direct reaction may persist, however, for some time after the serum bilirubin has fallen to within normal limits in some cases.

Hepatocellular Jaundice. This type of jaundice, sometimes called nonobstructive hepatic jaundice, is due to hepatic cell damage usually resulting either from infection, directly or indirectly, or from chemical agents.³⁸

In certain of the infectious diseases, such as simple or "catarrhal" jaundice, spirochetal jaundice (Weil's disease) and yellow fever, hepatic damage and jaundice are dominant features; in others (Table 12), hepatic involvement is an incident in the course of a generalized infection. In some instances, hepatocellular damage is accompanied and may be aggravated by excessive hemolysis, as in infections with hemolytic streptococci, staphylococci and pneumococci, malaria, *Clostridium perfringens*, and so on, and in favism.

Hepatotoxic chemical agents may act in one or more of three ways.³⁹

(1) Direct injury to liver cells ether, chloroform, carbon tetrachloride, avertin, carbon disulfide, sulfonamides, dinitrophenol, nitrobenzene, trinitrotoluene, tetrachlorethane, synthalin, cinchophens, acriflavine, arsenic, phosphorus, manganese, tannic acid, amanitotoxin and bean poison (favism).

(2) Hemolysis, with injury to liver cells, either directly or by products of hemolysis (red cell stroma): phenylhydrazine, sulfonamides, trinitrophenol, arsine, toluylenediamine, snake poisons, saponins, incompatible blood, hemolytic sera, bean poison.

(3) Hypersensitivity, idiosyncrasy, allergy: arsphenamines, cinchophens, mercury, bismuth, sulfonamides, liver extract, and a number of other agents employed for therapeutic purposes.

Hepatocellular damage, with jaundice, has been observed

Extrahepatic Obstructive Jaundice. In experimental animals, when the pressure in the bile ducts is increased to a level higher than the secretory pressure of the liver, bilirubin usually appears in the thoracic duct lymph in ten to twenty minutes and increases in the blood of the systemic circulation usually after twenty minutes.^{34,44} It appears probable that, as a result of the obstruction, bilirubin diffuses from the bile capillaries to the hepatic lymph spaces and from the latter to the adjacent blood capillaries. Barron² found that a negative direct van den Bergh reaction was obtained during the first two hours and a positive direct reaction in two to four hours after ligation of the common duct. He believes that the initial negative direct reaction may be due to temporary reflex inhibition of hepatocellular function comparable to the reflex anuria which at times follows ligation of one ureter. It may also be due to functional saturation of the hepatic cells with bilirubin and a diminished capacity for removing the pigment from the blood. The subsequent diffusion of direct-reacting bilirubin from the bile canaliculi into the lymph spaces and blood capillaries results in the production of the direct reaction in the blood serum. The accumulation of indirect-reacting bilirubin in the blood due to inability of the liver cells to remove it adequately constitutes one form of "retention jaundice," while diffusion of direct-reacting bilirubin from the bile canaliculi into the lymph and blood has been termed "regurgitation jaundice."⁴¹

TABLE 13
QUANTITATIVE DIRECT AND INDIRECT SERUM BILIRUBIN

Condition	Cases	Total bilirubin mg. %	Direct bilirubin			Indirect bilirubin	
			% of total	Mg. %	Increased %	Mg. %	Increased %
Normal	50	0.1-0.8	0-75	0-0.35	...	0.05-0.54	
Sulfonamide poisoning	32	0.4-10	8-79	2-3.8	87	.2-6.2	75
Hepatitis	172	2-20	19-100	.2-18	66	0-4.2	80
Cirrhosis	41	4-10	31-100	36-4.2	100	0-6.2	56
Extrahepatic biliary obstruction	75	5-36	50-93	2.6-32	100	2.4-8.4	100
Congestive heart failure	46	7-5	26-64	.19-2.8	94	.51-2.2	94
Pernicious anemia	6	9-2	34-67	14-16	100	.3-1.7	40
Hemolytic jaundice	3	1.4-9.5	3-14	.2-3	0	1.2-9.2	100

most important of which are enumerated in Table 12 (p. 439). It is probable that normal hepatic cells are capable of handling efficiently much more bilirubin than they are called upon to excrete under normal circumstances. For the production of hyperbilirubinemia due to excessive hemolysis, it is probably essential that there be also some degree of impairment of hepatic cell function. This functional insufficiency is believed to be due usually to anoxemia, which is present constantly in such conditions. In uncomplicated cases, and in the absence of severe morphologic damage to the liver cells, the serum gives a negative direct van den Bergh reaction. However, as indicated previously (p. 441) and in Table 11 (p. 438), severe anoxia, or the products of hemolysis, especially the erythrocyte stroma material, may cause serious hepatocellular damage with the production of a superimposed regurgitation type of jaundice and a positive direct van den Bergh reaction in the serum. In congenital hemolytic jaundice (spherocytic jaundice), the rather common complication of biliary calculi may be responsible for the same phenomenon.

Serum bilirubin values above 6 mg. per 100 cc. are unusual and above 10 mg. rare in uncomplicated hemolytic jaundice. It has been found that in icterus neonatorum⁵⁴ and congenital hemolytic jaundice⁵ the increase in serum bilirubin is entirely in the nondirect-reacting form of pigment. In pernicious anemia, in which hyperbilirubinemia is probably not, strictly speaking, due to excessive hemolysis, there is a more consistent increase in direct-reacting than in nondirect-reacting bilirubin, although the qualitative direct reaction is invariably negative in this condition in the absence of complications.

BILIRUBINURIA

As stated above, bilirubin giving a direct van den Bergh reaction is apparently more diffusible than that giving only the indirect reaction. In the case of the former, with bilirubinemia above 1.6 mg. per 100 cc., the so-called "threshold value," the pigment passes through the blood capillary walls into the tissues and urine. In the case of the latter, the threshold for passage into the tissues is higher and the renal threshold value usually much higher than 1.6 mg. per 100 cc. Consequently, clinical icterus usually appears later in hemolytic than in obstructive jaundice, and bilirubinuria may be absent in spite of relatively high grades of hyperbilirubinemia. In congenital hemolytic jaundice, serum bilirubin concentrations as high as 9 mg. per 100 cc. have been observed without bilirubinuria.^{2,52} There is some evidence that the passage of direct-reacting bilirubin into

after x-ray overdosage and in eclampsia and severe burns (tannic acid therapy?). This type of hyperbilirubinemia may occur also occasionally in diabetes mellitus and rather commonly in congestive heart failure, erythroblastosis fetalis and cirrhosis of the liver. In erythroblastosis fetalis, and probably in congestive heart failure, it is contributed to by increased bilirubin formation resulting from excessive hemolysis.²⁶

The essential feature of hepatocellular jaundice is functional insufficiency of the hepatic cells. Under normal conditions bilirubin diffuses from the hepatic sinusoids into the perivascular spaces and is taken up by the hepatic cells and passed into the bile canaliculi. In the presence of hepatocellular damage the liver cells are unable to remove the required amount of bilirubin, which consequently accumulates in the blood. At this stage the direct van den Bergh reaction is negative, since, theoretically, at least, there has been no increase in direct-reacting bilirubin. As the condition progresses, hepatic cell damage increases (degeneration, necrosis, desquamation), the continuity of the bile canaliculi is disrupted and their lumen may be occluded by desquamated and disintegrated cells (bile thrombi). As a result, some bilirubin which has entered the canaliculi may pass back into the perivascular spaces and blood capillaries, with the production of a positive direct van den Bergh reaction in the blood serum.² In all but very mild cases of hepatocellular jaundice, the reaction is therefore practically identical with that in obstructive jaundice, differing however, in this respect: at the onset of the disease, the negative direct reaction may persist for a longer time than in obstructive jaundice. As indicated in Table 13, in hyperbilirubinemia of this type, as exemplified by hepatitis, cirrhosis, congestive heart failure and sulfonamide poisoning, there is usually an increase in both direct and nondirect-reacting bilirubin, as in obstructive jaundice, indicating the operation of both retention and regurgitation mechanisms in its pathogenesis.

A familial type of jaundice has been described¹¹ with serum bilirubin concentrations as high as 13 mg. per 100 cc. and a negative direct van den Bergh reaction, in which the capacity of the hepatic cells for excreting bilirubin seems to be impaired ("constitutional hepatic dysfunction"). There is no evidence of excessive hemolysis or hepatic disease, nor of any impairment of liver function other than that of excreting bilirubin. A condition of "hereditary jaundice" of apparently identical nature has been described in rats.³²

Hemolytic Jaundice. This is observed clinically as a result of excessive hemolysis in a number of conditions, some of the

most important of which are enumerated in Table 12 (p. 439). It is probable that normal hepatic cells are capable of handling efficiently much more bilirubin than they are called upon to excrete under normal circumstances. For the production of hyperbilirubinemia due to excessive hemolysis, it is probably essential that there be also some degree of impairment of hepatic cell function. This functional insufficiency is believed to be due usually to anoxemia, which is present constantly in such conditions. In uncomplicated cases, and in the absence of severe morphologic damage to the liver cells, the serum gives a negative direct van den Bergh reaction. However, as indicated previously (p. 441) and in Table 11 (p. 438), severe anoxia, or the products of hemolysis, especially the erythrocyte stroma material, may cause serious hepatocellular damage with the production of a superimposed regurgitation type of jaundice and a positive direct van den Bergh reaction in the serum. In congenital hemolytic jaundice (spherocytic jaundice), the rather common complication of biliary calculi may be responsible for the same phenomenon.

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the urine and possibly also into the tissues is dependent upon the usual simultaneous presence of an increase in bile salts in the blood (obstructive and hepatocellular jaundice)^{1,65} rather than upon an inherent difference in the diffusibility of the pigment molecules.

As stated by Naumann, the ordinary tests for bilirubin in the urine, depending upon the formation of biliverdin or bilicyanin, are only transitory stages of bilirubin oxidation, the final stage being represented by the pink choletelin, which is formed more rapidly in the presence of small amounts of bilirubin. This author suggests a method for detecting bilirubin in urine which consists in adsorbing the urinary pigment on a layer of talc and producing blue bilicyanin by oxidation with a drop of Fouchet's reagent or 10 per cent nitric acid. It is possible by this method to detect bilirubin in urine in a concentration of 0.009 parts per million. Applying this method, bilirubin has been found in normal urine, quantitative estimation by means of a dilution technic yielding values of approximately 0.3 mg. per 100 cc., about the same as the bilirubin content of blood plasma. This is in accord with the view that the concept of a so-called threshold for bilirubin elimination by the kidneys is fallacious.

UROBILIN AND UROBILINOGEN IN THE URINE AND FECES

The relationship between the various derivatives of hemoglobin is illustrated in Fig. 16 (p. 431), and has been described previously (p. 430). The pertinent facts regarding the metabolism of urobilinogen may be summarized briefly as follows.

Under normal conditions bilirubin entering the intestine in the bile is broken down through the action of putrefactive organisms into urobilinogen (reduction), a colorless substance, which, through a process of oxidation, is transformed into urobilin (stercobilin). Urobilin and urobilinogen are present in the stools of normal individuals and, in traces, in both bile and urine. A portion of the urobilinogen formed in the intestine is reabsorbed into the portal blood stream and carried to the liver where it is reconverted into bilirubin or some other pigment complex. This cycle is termed the enterohepatic circulation of bile pigment. The following conclusions may be drawn from the work of Elman and McMaster (Rolleston and McNee):

(1) Bacterial infection or putrefaction is necessary for the conversion of bilirubin to urobilin. This may occur in the intestine (normal) or in the biliary passages in the presence of infection.

(2) In conditions of moderate hepatic damage, incomplete biliary obstruction, cholangitis and hemolytic jaundice the liver is unable to metabolize completely all of the bilirubin absorbed from the intestine and some consequently passes into the general circulation and is eliminated in the urine. Under such circumstances urobilinuria is an indication of defective hepatic function. In pure hemolytic icterus, although the function of the liver may be unimpaired, it cannot metabolize completely the excessive amounts of bilirubin formed from the increased quantity of bilirubin which passes into the bile.

Urobilinogen in Feces. The accurate quantitative estimation of the urobilinogen content of the feces is seldom employed clinically but may be of great value in certain conditions. Watson, using an improved quantitative method, found the normal daily excretion in the feces of adults to range from 40 to 280 mg. (usually 100–250 mg.). The urobilinogen content of the feces or duodenal contents appears to be an index of the degree of blood destruction in the absence of hepatic or biliary tract disease. Increased quantities have been found in the duodenal bile and feces in hemolytic jaundice, paroxysmal hemoglobinuria and other hemolytic processes (Table 12, p. 439) and during periods of relapse in pernicious anemia. In the latter condition, during remissions induced by liver therapy, the urobilin content of the feces drops to within normal limits.

Low values may be obtained in the presence of inanition, inactivity, low-grade infections, hypochromic anemia and certain cases of secondary hyperchromic anemia. Inasmuch as the quantity of urobilinogen in the feces depends primarily upon the quantity of bilirubin entering the intestine, variations in the former in hepatic and biliary tract disease are determined largely by (1) the degree of impairment of bilirubin excretion by the hepatic cells, (2) the presence and degree of obstruction to the flow of bile and (3) the presence and severity of an associated hemolytic process.

This determination may be of value in distinguishing between jaundice due to stone in the common duct and that due to neoplasm of the duct, duodenum or head of the pancreas. Theoretically, in the absence of marked biliary tract infection, complete obstruction of the common duct, with consequent absence of bile pigment from the intestine, should result in a total absence of urobilinogen from the feces and urine. This fact is applied particularly to the differentiation between common duct obstruction due to stone, which is seldom permanently complete, and that due to neoplasm, which usually becomes permanently complete. Watson found that absence of uro-

bilinogen from the urine, a quite constant finding in obstructive jaundice due to neoplasm, was also noted at times with varying degrees of obstruction due to stone. However, he found that the excretion of urobilinogen in the feces differed strikingly in these two groups of patients, being frequently within normal limits (10-250 mg. daily) in the patients with calculous obstruction, and usually low or extremely low or absent (0-5 mg. daily) in those with malignant obstruction.

Urobilinogen in Urine. As stated above, absence of urobilinogen from the urine in patients with jaundice is indicative of complete obstruction of the common duct or, of complete suppression of bile pigment excretion by the liver. This finding may also be present, whether or not jaundice is present, in patients with bile fistula in whom the bile is draining either externally or into some viscus other than the intestine. However, some urobilinogen may be present in the urine occasionally in cases of complete obstruction of the common duct. This may occur particularly in cases of calculous obstruction with marked infection of the bile passages and occasionally in patients with marked jaundice in whom some bile pigment may pass into the lumen of the bowel directly from the jaundiced intestinal mucous membrane.

Watson has found that 1 to 4 mg. of urobilinogen are eliminated daily in the urine by the normal adult. According to the less accurate methods commonly employed (Wallace and Diamond), a positive reaction for urobilinogen may be obtained normally with urine dilutions up to 1 to 20; a positive reaction with dilutions above this figure is considered indicative of the presence of excessive quantities of urobilin bodies. Although in pathologic conditions the morning urine may contain relatively large quantities of urobilinogen, the rate of elimination varies so much from hour to hour and day to day that all estimations should be made on twenty-four-hour specimens and should be repeated for at least five consecutive days before a negative reaction is interpreted as indicating the absence of urobilinuria.

Excessive amounts of urobilinogen and urobilin are found in the urine under the following circumstances:

Excessive Hemolysis. Excessive urobilinuria occurs characteristically in congenital and acquired hemolytic jaundice, pernicious anemia, splenic anemia and other conditions associated with hemolytic features (Table 12, p. 439), due to the fact that the liver is unable to deal with the excessive amount of urobilinogen formed from the abnormally large quantity of bilirubin in the intestine. A sudden increase in urine urobilinogen has been observed shortly after the occurrence of pulmonary

infarction in patients with congestive heart failure or auricular fibrillation, due presumably to hemolysis in the infarcted areas. It has been found that patients with pernicious anemia in whom excessive urobilinuria disappears before the reticulocyte crisis (induced by treatment) exhibit a greater increase of hemoglobin and red blood cells than those in whom excessive urobilinuria persists for even a short time after the reticulocyte crisis. Some believe that excessive urobilinuria in these anemic conditions is due in part to some disturbance of hepatic function, attendant perhaps upon the existing state of anoxemia. Similar findings have been obtained during malarial chills. It is believed by some that the total urobilinogen excretion may be regarded as a quantitative index of the amount of hemoglobin destruction (p. 432).

Hepatic Functional Impairment. (a) HEPATOCELLULAR JAUNDICE. Urobilinuria occurs regularly in the presence of hepatic functional impairment as long as adequate quantities of bilirubin are entering the bowel. This phenomenon occurs, therefore, in the great majority of patients with jaundice due to parenchymal hepatic lesions, including hepatitis, acute and subacute hepatic necrosis, toxemia of pregnancy, arsphenamine, chloroform and carbon tetrachloride poisoning, congestive heart failure and liver damage due to a great variety of other toxic and infectious agents. In animals poisoned with chloroform, it has been found that if the hepatic damage is so severe that no bilirubin is excreted by the liver, urobilinuria does not occur. This finding coincides with the common clinical observation of excessive urobilinuria in the very early and late stages of acute hepatitis, including so-called catarrhal jaundice, while it is absent during the stage of complete suppression of bilirubin excretion or obstruction to its excretion due to cholangitis. We have observed excessive urobilinuria in the early stages of acute and subacute hepatic necrosis which disappeared as the condition progressed and the degree of bilirubinemia increased with the development of such marked hepatocellular damage that no bile pigment was eliminated.

Many observers believe that excessive urobilinuria is perhaps the most sensitive single index of the presence of liver dysfunction. It frequently persists after all other evidence of hepatic damage has disappeared and may appear before the development of any other manifestation of such damage.

(b) HEPATIC DISEASE WITHOUT JAUNDICE. Excessive urobilinuria occurs commonly in portal cirrhosis even in the early stages of its development. In this condition, it is due in part to hepatic functional impairment and, in addition, to the fact

that a portion of the portal blood reaches the general circulation without having passed through the liver. In this condition, excessive urobilinuria may occur in the absence of hyperbilirubinemia. Urobilinuria has been frequently observed in patients with congestive heart failure, considerable prognostic significance being attributed to it by some authors. In this condition, it is probably dependent upon the presence of hepatic damage, functional or organic, resulting perhaps partly from a state of prolonged anoxemia incident to the existing circulatory disturbance, and partly from injury to the polygonal cells by pressure from the dammed-back blood. It usually precedes the development of hyperbilirubinemia. It may also be observed in the absence of jaundice in certain toxic states, particularly pneumonia, streptococcus infections and malaria, due partly to excessive hemolysis and partly to hepatic parenchymal damage. Because of the nature of the conditions responsible for its occurrence, excessive urobilinuria is regarded by some as of great prognostic significance in pneumonia. This finding has also been obtained in patients with cholecystitis and cholelithiasis in the absence of hyperbilirubinemia, due to an associated mild hepatitis. This determination is of particular value, therefore, in the preoperative study of such patients.

(c) **OBSTRUCTIVE JAUNDICE.** It now appears that most of the discordant experimental observations regarding the question of the formation of urobilinogen in the absence of bilirubin from the intestine may be explained on the basis of the presence or absence of active infection of the bile passages in the experimental animals. The work of Elman and McMaster has demonstrated rather conclusively that, except in the presence of such infection, urobilinuria rarely if ever occurs in complete obstructive jaundice. As was mentioned, in some cases of profound jaundice, a small quantity of urobilinogen may be formed from bile pigment which has passed from the jaundiced intestinal mucosa into the lumen of the bowel. It has been shown experimentally that urobilin bodies are not present in the urine in complete biliary obstruction even though the liver be simultaneously severely damaged by chloroform, phosphorus or other hepatotoxic agents. It has frequently been observed that urobilinuria, occurring in patients with cholelithiasis, disappears completely with the development of complete obstruction of the common bile duct. When the bile flow is reestablished, urobilinuria reappears. This phenomenon is observed not only in cholelithiasis but also in hepatitis with associated cholangitis, as in so-called "catarrhal jaundice." Urobilinuria may be observed in infective cholangitis, especially when a gallstone is floating in

the common duct. It may also be encountered occasionally in patients with complete calculous obstruction complicated by purulent cholangitis. Such cases are, however, not common, and it may be assumed generally that in the majority of cases of obstructive jaundice the presence of urobilinogen in the urine suggests that the obstruction is not permanently complete. Under such circumstances, excessive urobilinuria is frequently encountered, dependent upon associated marked hepatic functional impairment with consequent inability of the liver to remove from the blood even relatively small quantities of urobilinogen absorbed from the bowel.

As stated by Rolleston and McNee, urobilinuria is not pathognomonic of any single morbid condition, its chief diagnostic significance, apart from its occurrence in hemolytic anemia, pernicious anemia and chronic hemolytic jaundice, being that some bile pigment is entering the intestine and that the functional activity of the liver is either very considerably impaired or that there is active infection in the bile ducts. When properly interpreted, the study of urobilinogen excretion is of great value not only in the study of the state of hepatic function but also in differentiating between complete and incomplete obstruction to the flow of bile. This may be of distinct value in distinguishing between jaundice due to stone in the common duct and that due to neoplasm of the duct, duodenum or head of the pancreas. In the latter connection, simultaneous investigation of the urobilinogen excretion in the feces is of great value (p. 415).

THE EXCRETION OF INTRAVENOUSLY INJECTED BILIRUBIN (Bilirubin Tolerance)

It is well recognized that there are conditions of mild liver injury without associated hyperbilirubinemia, this being possible either because the liver cells are still able to eliminate the normally circulating bilirubin or because the production of this pigment is diminished. On the basis of the assumption that hepatic insufficiency might be demonstrated by injecting an additional amount of bilirubin and studying the rate of its elimination, this procedure has been suggested as a test of hepatic functional efficiency.^{15,23,25,46,47,50} The rationale of this test rests upon the apparently well-founded belief that bilirubin, injected intravenously, is not excreted through the kidneys nor retained by the cells of the reticulo-endothelial system but that it is totally excreted by the liver.

It has been found that when bilirubin is injected intra-

venously, into normal individuals in a dosage of 1 mg. per kilogram of body weight, it is practically completely excreted from the blood stream in from two to four hours. If more than 6 per cent of the injected bilirubin is retained in the blood at the end of four hours, the ability of the liver to excrete the pigment may be considered to be impaired. With a dosage of 1.5 mg. per kilogram of body weight, retention in excess of 15 per cent at the end of four hours is abnormal. Studies in selected cases of slight liver injury demonstrate that it is possible to recognize insufficiency of the liver by this method when all of the commonly used procedures fail to give evidence of hepatic damage (e.g., cirrhosis, chronic hepatitis, congestive heart failure, severe anemia). The study of the bilirubin excretory power of the liver is probably the most delicate method thus far proposed for testing the excretory functional capacity of this organ.

This test should only be applied in cases in which the serum bilirubin concentration is within normal limits, for, in the presence of hyperbilirubinemia, no further information can be obtained by increasing the amount of pigment already present in excess in the blood. In apparent contradiction of this statement, however, it has been reported that normal results may be obtained with this procedure in certain cases of hemolytic jaundice.^{15,23}

BILE PIGMENTS IN FECES

Inasmuch as bilirubin is practically completely converted to urobilinogen and stercobilinogen in the intestine, the latter constitute the normal pigment of the feces, bilirubin being rarely present, except occasionally in severe diarrhea with extremely rapid passage of material through the intestine, and in gastrocolic fistula. The persistent absence of pigment from the feces is indicative of obstruction to the outflow of bile from the liver or of complete suppression of bile pigment excretion by the liver. Complete obstruction of the bile passages may occur in cholangitis but is most commonly associated with stone in the common duct, stricture of the bile ducts, carcinoma of the head of the pancreas, common duct or duodenum or, rarely, enlargement of the lymph nodes in the region of the common bile duct. The feces are pale, having so-called "clay-colored" or putty-like appearance, and are usually bulky, extremely offensive and contain excessive amounts of neutral fat and fatty acid (p. 428). If the obstruction is not complete the feces may be fairly normal in color and, as demonstrated by Elman and McMaster, even in complete obstruction some urobilinogen may be present in the feces due to the entrance of small amounts of bile pigment into

the intestine from the capillaries of the intestinal mucous membrane.

The significance of accurate quantitative estimations of the urobilinogen content of the feces in this connection has been referred to elsewhere (p. 445). It must be remembered, too, that so-called "clay-colored" feces may actually contain considerable amounts of pigment, the presence of which is obscured by large amounts of fat. Before deciding that pigment is absent from the feces under such circumstances, the fat should be removed by extraction. Of greater value in determining whether or not bile is entering the intestine is the procedure of duodenal intubation, the tube being allowed to remain *in situ* for periods of several hours on successive days.

PORPHYRIN IN URINE AND FECES

The pertinent facts regarding normal porphyrin excretion have been outlined elsewhere (p. 432). Under normal conditions the daily excretion of coproporphyrin is about 10-120 micrograms in the urine and 100-200 micrograms in the feces, the normal urine: feces ratio being about 0.1-0.6.

Increased porphyrinuria has been reported in patients with cirrhosis, hemochromatosis, hepatitis, melanosarcoma of the liver, acute and subacute hepatic necrosis, chronic passive congestion of the liver, obstructive jaundice, catarrhal jaundice and metastatic carcinoma of the liver. This is due largely to inability of the liver to excrete the porphyrin in the bile. In the absence of biliary obstruction, an increase in the urine: feces porphyrin ratio may be of more significance in indicating impairment of hepatic function than the absolute amount excreted in the urine.^{29,37} This ratio has been found to be 0.8-22.0 in patients with hepatic disease.

Increased urinary and fecal excretion of coproporphyrin (and uroporphyrin) occurs also in congenital porphyrinuria, an "inborn error of metabolism," and occasionally in lead poisoning, pernicious anemia, hemolytic anemias, hemolytic jaundice, acute idiopathic porphyrinuria, after administration of excessive amounts of quinine, trional, sulfonal and barbital, and in certain infectious diseases (tuberculosis, pneumonia, rheumatic fever). The pigment may also be present in serous effusions, bones and teeth, and the skin may be abnormally sensitive to ultraviolet light in the congenital form, in which the condition is inherited possibly as a mendelian recessive, appears early in life and is characterized by excessive excretion, particularly of uroporphyrin I, in the urine.

Acute idiopathic porphyria differs from the above type of

congenital porphyria in that it is possibly inherited as a mendelian dominant, it appears usually late in life, photosensitivity is rare, pigmentation of the teeth does not occur and the predominating pigment is almost always uroporphyrin III, excreted in the urine as a metal complex (zinc), accompanied by uroporphyrin I and, at times, an excess of coproporphyrin.

BILE ACID METABOLISM

The bile acids, glycocholic and taurocholic acids, are present in the bile and blood as their sodium salts, the relative proportions of the two substances varying in different individuals. Very little is known regarding the origin of these substances. However, it is now known that the fundamental ring systems of cholesterol and the bile acids are identical, suggesting an intimate metabolic relationship between these two substances. Recent studies in this field have revealed the interesting fact that an apparently unconnected series of physiologically highly active substances, such as sex hormones, calciferol (crystalline vitamin D), cholesterol, bile acids, cardiac glucosides and certain carcinogenic hydrocarbons, have essentially the same fundamental chemical nature, being constructed about a cholane nucleus.

In spite of the absence of direct evidence, there is considerable evidence of an indirect nature suggesting that the bile acids, whatever may be their origin, are formed in the liver. Following their elimination in the bile they are absorbed from the intestine and returned to the liver in the portal circulation. This cycle has been termed the enterohepatic circulation of bile acids.² Very little is known concerning the mechanism which controls the production and destruction of bile acids in the body, a fact which is due largely to the lack, until recently, of a satisfactory method for their quantitative estimation in the blood serum and in the bile. Recent studies by approved methods indicate that the liver, as well as being the site of formation of bile salts, plays a dominant role in their destruction.^{1,8} The liver appears to destroy about half of the bile salts which it forms daily. The chief physiologic function of the bile salts appears to be dependent upon their remarkable power of lowering surface tension, by virtue of which property they aid greatly in the emulsification of fats in the intestine, thereby facilitating their absorption (p. 132). In addition they exert a distinct cholagogue and choloretic effect. It has also been shown that the presence of bile acids is necessary for the absorption of carotene (pro-vitamin A) and vitamin D from the intestine.^{9,10}

It has been reported that normal blood yields a Pettenkofer

value equivalent to 3 to 6 mg. of glycocholic acid per 100 cc. Increased values, ranging from 7.2 to 16.7 mg. per 100 cc., have been observed in hepatic disorders, including carcinoma of the liver, portal and biliary cirrhosis and obstructive jaundice. However, recent observers, employing the more satisfactory method of Gregory and Pascoe, have found little or no bile acid in the blood of normal subjects. Because of technical difficulties, the quantitative estimation of bile salts in the blood is not commonly employed in the study of patients with hepatic or biliary tract disease. It has been found, however, in clinical and experimental obstructive jaundice, that bile salts appear in the blood and increase for some time during the early period of stasis.¹ After prolonged obstruction the concentration of bile salts in the blood diminishes, due presumably to diminished synthesis of these substances as a result of progressive hepatocellular damage. A similar secondary decrease in the bile salt concentration of the blood in animals with complete biliary obstruction has been observed following the administration of hepatotoxic substances, such as chloroform, carbon tetrachloride and tetrachlorethane. Although there have been occasional reports of an increase in bile salts in the blood of patients with portal cirrhosis, carcinoma of the liver and other hepatic diseases, these findings have not been substantiated by recent clinical and experimental studies. All available evidence indicates that the concentration of these substances in the blood increases only in the early stages of biliary stasis and not in the presence of hepatocellular damage in the absence of obstruction to the flow of bile through the extrahepatic bile passages.

From a practical standpoint, alterations in bile salt metabolism are investigated chiefly by determining the presence or absence of these substances in the urine and their concentration in the bile, the latter particularly in patients with bile fistula. In obstructive jaundice, the increase in the bile salt concentration of the blood is accompanied by the appearance of the salts in the urine. The development, in such cases, of marked hepatocellular damage, whether due to prolonged stasis, superimposed hepatitis or the administration of hepatotoxic agents, is followed by a fall in the bile salt concentration of the blood and, in some cases, a disappearance of bile salts from the urine. The presence of bile pigment in the urine in the absence of bile salts is termed dissociated jaundice. The same phenomenon, constituting another type of dissociated jaundice, occurs occasionally in patients with hemolytic icterus.

The importance of certain abnormalities in the composition of bile is discussed elsewhere (p. 467). From a practical stand-

point, investigation of the bile salt content of bile may be of value in patients with bile fistula or with a drainage tube in the common duct following operation upon the bile passages. Ravdin has found that bile salts are invariably absent from the liver bile in cases in which the common bile duct has been obstructed for a week or more. A period of one to four weeks or longer may elapse between the time of release of obstruction and the re-appearance of bile salts in the liver bile. This interval appears to be roughly proportional to the degree and duration of obstruction and the degree of cholangitis associated with the obstruction. The persistent absence or very low concentrations of bile salts in the drainage bile under such circumstances is of poor prognostic significance, indicating severe hepatocellular damage. The observations of Bollman and Mann suggest that the absence of bile salts from the bile in such cases is due to failure of synthesis of these substances by the liver rather than to their increased destruction. The serious significance of this phenomenon is indicated by the fact that the liver is able to synthesize bile acids in essentially normal amounts even in the face of extensive hepatic damage, as in advanced portal cirrhosis. There is some evidence that the failure to demonstrate bile salts in the bile under such circumstances is due to the presence of abnormal forms, perhaps representing intermediate stages in the synthesis of cholic acid, which do not respond to the Pettenkofer reaction.

DETOXIFICATION-CONJUGATION

The liver is believed to play an important part in protecting the organism from various toxic substances entering through the intestinal tract. This detoxifying function is believed to involve processes of oxidation and conjugation into relatively nontoxic substances which are subsequently eliminated in the bile and urine. For example, indol absorbed from the intestine reaches the liver in the portal circulation, is oxidized into indoxyl, conjugated with sulfuric acid and eliminated in the urine as indoxyl sulfuric acid. Other substances, including salicylic acid, menthol, camphor, phenol, etc., are in this way transformed into conjugate glucuronates and excreted in the urine. The estimation of inorganic and ethereal sulfates in the blood and urine and of conjugate glucuronates in the urine following the administration of such substances has been proposed as a method of estimating liver function. These tests have proved to be of little clinical value.

In recent years, investigation of the ability of the organism to synthesize hippuric acid and to oxidize cinchophen has been

proposed for the study of the detoxifying and conjugating activity of the liver. The hippuric acid excretion test has been widely employed and has proved to be one of the most satisfactory methods of studying hepatocellular function.

Hippuric Acid Synthesis.^{39,40,45,56} This test is based upon the ability of the organism to conjugate glycine and benzoic acid to form hippuric acid, which is eliminated in the urine. The site of synthesis of hippuric acid has been the subject of considerable controversy; early experimental observations suggested that it has its origin in the kidneys. The work of Quick suggests that in man the liver is perhaps the principal site of formation of this substance. It was found that the hourly rate of excretion of hippuric acid in the urine of normal subjects following the ingestion of benzoic acid is remarkably constant, being influenced somewhat by the body surface area. In certain types of hepatic disease the output of hippuric acid is markedly reduced. This reduction is assumed to be due in part to a diminished capacity of the liver to synthesize glycine, which is essential for the formation of hippuric acid, and in part to damage of the enzymatic mechanism which unites benzoic acid with glycine. In man, this conjugating enzyme appears to be present chiefly in the liver, and the small amount which is present in the kidneys is regarded as insufficient to compensate for the hepatic defect in liver disease. The urinary elimination of hippuric acid after the ingestion of benzoic acid is therefore regarded as a measure of the capacity of the liver to furnish glycine (amino-acetic acid) and also as an index of its detoxifying or conjugating power.

A subject with a normally functioning liver excretes approximately 3 Gm. of benzoic acid, in the form of hippuric acid (4.41 Gm), in the urine in four hours after the ingestion of 6 Gm. of sodium benzoate. The normal range of variation has been established at 85-110 per cent of this figure. In many cases the intravenous route of administration of the sodium benzoate is preferable in order to obviate the possibility of error due to abnormalities of absorption from the intestine. Normal subjects excrete 0.7-0.95 Gm. of benzoic acid (as 1.0-1.4 Gm. hippuric acid) in the urine during the first hour after intravenous injection of 1.77 Gm. of sodium benzoate. It has been found that the excretion of hippuric acid under these circumstances is influenced by the size of the subjects and the following equation has been suggested for calculating the predicted normal excretion:⁴³

$$\text{Hippuric Acid (grams)} = 0.34 + (0.00668 \times \text{weight in pounds}).$$

Subnormal values have been obtained in patients with a variety of hepatic disorders, including catarrhal jaundice, various other forms of hepatitis, hepatic syphilis, portal cirrhosis, biliary cirrhosis, metastatic carcinoma of the liver and acute and subacute hepatic necrosis. It has been suggested that hyperexcretion of hippuric acid (120-165 per cent of normal) may occur in mild forms of hepatic disease, as in hepatitis accompanying cholecystitis. Normal values have been obtained in uncomplicated obstruction of the common duct, abnormal findings being observed in cases of extremely long duration and in those with superimposed hepatocellular damage. The test may, therefore, be of value in differentiating between hepatocellular and obstructive types of jaundice.

Several authors have confirmed the value of this procedure in the study of hepatic function; however, it is necessary to investigate the state of renal function before attaching significance to abnormal findings in patients with hepatic disease. This is due to the fact that diminished or less rapid elimination of hippuric acid may be dependent upon impairment of kidney function, which is present frequently in such cases. It has been found also that synthesis and excretion of hippuric acid are subnormal in conditions other than nephritis and hepatic disease, among these being cachectic states, anemia and chronic passive congestion. If the presence of these disturbing factors can be excluded, this test is, in our experience, one of the most useful of the available methods of studying hepatocellular function.

ELIMINATION OF DYES¹

It has been found that certain dyes are removed from circulation and eliminated almost entirely by the liver, just as phenolsulfonephthalein is excreted almost entirely by the kidneys. Among these dyes are azorubin S., Rose Bengal, phenoltetrachlorophthalein, phenoltetraiodophthalein and sodium phenoltetrabromphthalein sulfonate (bromsulfalein). Several liver function tests have been proposed which have for their basis the estimation of the ability of the liver to eliminate these substances. These tests, as originally described, consisted in the determination of the quantity of dye recovered from the duodenal contents and feces following its injection into the blood stream. This method has now been largely abandoned, the procedure in common use at the present time consisting in the determination of the rate of removal of the dye from the blood stream, the disappearance rate being assumed to be an indication of the state of hepatic function.

Bromsulfalein Excretion. Bromsulfalein has proved to be most satisfactory because it is less toxic and much less irritating than most of the other dyes which have been proposed. It has been noted repeatedly that the phthalein dyes leave the blood stream within a few hours after their intravenous injection in clinical dosage, regardless of the condition of the liver. However, careful examination of animals with obstruction of the common duct has frequently failed to reveal any evidence of accumulation of the dye in the tissues or in the contents of the obstructed bile passages, despite the fact that relatively insignificant quantities had been eliminated in the urine and little or none was present in the blood. There is some evidence that the removal of bromsulfalein from the blood may be related to reticulo-endothelial cell activity. Presumably, then, abnormal retention of the dye in hepatic or biliary tract disease might be attributed to impaired Kupffer cell function. This theory is in harmony with the observation that the dye disappears from the blood of normal animals more rapidly than it is eliminated in the bile. Theoretically, therefore, bromsulfalein retention may be due either to dysfunction of the reticulo-endothelial system, defective hepatic excretion or both. It is believed by some that considerable amounts of the injected dye may undergo destruction in the tissues, accounting for the phenomenon referred to above.

Following intravenous injection of 2 mg. of bromsulfalein per kilogram of body weight, in normal subjects, 20-60 per cent remains in the blood serum or plasma after five minutes, 0-10 per cent after fifteen minutes and none after thirty minutes. When 5 mg. per kilogram are injected, the corresponding values are 30-85 per cent at five minutes, 0-30 per cent at fifteen minutes and 0-10 per cent at thirty minutes.^{3b, 7a} In the great majority of cases, the determination of thirty-minute retention suffices for purposes of routine study. The 2 mg. dosage possesses the advantage that any retention of dye after thirty minutes is abnormal. The advantage of the 5 mg. dosage lies in the fact that it imposes a greater functional burden upon the hepatic cells and, consequently, may yield abnormal results in cases of mild functional impairment in which normal results may be obtained with the 2 mg. dosage. Similarly, readings made at five and fifteen minutes may reveal abnormal retention in cases of mild functional impairment in which the thirty-minute values may be normal.

Recent studies of the curve of elimination of bromsulfalein in the bile (duodenal intubation) suggest that this method of investigation may yield valuable information in some cases in which the rate of removal of dye from the blood may be nor-

mal.^{3a,15} Under normal conditions, with the 2 mg. dosage, the dye usually appears in the bile within five to fifteen minutes, its excretion continuing over a period of two to six hours. Fifty to 85 per cent of the quantity injected is excreted in the bile in one hour and 65-100 per cent in two hours, the curve of excretion reaching a maximum level at forty-five to seventy-five minutes and falling to a relatively low level at two hours. These findings suggest that the dye is removed rapidly from the blood and is subsequently excreted more slowly in the bile.

Abnormalities of the mechanism for removing bromsulfalein from the body may be evidenced by one or more of the following phenomena: (1) abnormal retention in the blood at five, fifteen or thirty minutes after injection; (2) delayed appearance of dye in the bile; (3) delayed attainment of maximum concentration in the bile; (4) prolonged high curve or low flat curve of biliary excretion of the dye; (5) subnormal total excretion in the bile at one or two hours after injection. In some cases, especially with incipient biliary obstruction or mild hepatic functional impairment, disturbances may occur in the "biliary" phase of excretion in the absence of abnormal dye retention in the blood.

*Extrahepatic Biliary Obstruction.*¹ In the presence of complete obstruction of the common duct, the degree of retention of the dye increases progressively with increasing bilirubinemia until all of the dye injected remains in the blood at the end of thirty minutes (2 mg. dosage). In the presence of complete obstruction, therefore, little information is obtained by this method which cannot be obtained by means of the quantitative determination of serum bilirubin. However, in incomplete obstruction, evidence of superimposed hepatocellular damage may be manifested by retention of bromsulfalein out of proportion to the degree of hyperbilirubinemia; it is of particular value, therefore, in the preoperative study of patients with cholelithiasis. It has been found that similar degrees of retention of dye may occur in obstructive jaundice due to gallstones at lower levels of bilirubinemia than in obstruction due to carcinoma of the pancreas. It is believed that in many patients with cholelithiasis and common duct obstruction due to stone, superimposed upon the conditions common to all cases of obstructive jaundice are those associated with chronic biliary tract disease, which may include hepatic functional impairment. It has also been observed that following relief of the obstruction, dye retention, although diminishing, frequently persists for a variable period of time after the serum bilirubin concentration has returned to normal. This is due perhaps to the residual hepatitis

which is present in nearly all patients who have suffered from biliary obstruction for an extended period.

*Hepatocellular Damage.*¹ Abnormal retention of bromsulfalein in the blood occurs almost invariably in acute hepatic disease, the degree of retention being roughly indicative of the extent of hepatic functional impairment. This is true of all varieties of hepatitis and acute and subacute hepatic necrosis due to any cause. The degree of dye retention usually, but not invariably, parallels the degree of hyperbilirubinemia in such cases. As in the case of obstructive jaundice, abnormal dye retention may occur before and may persist longer than hyperbilirubinemia. Some observers believe, therefore, that this test is of value in the early detection of hepatocellular damage in patients receiving hepatotoxic agents for therapeutic purposes, such as arsphenamine and carbon tetrachloride.

Varying degrees of dye retention, up to 100 per cent, may be obtained in patients with chronic hepatic lesions. Among these are chronic hepatitis, portal cirrhosis, biliary cirrhosis, malaria, hepatic syphilis, carcinoma of the liver and chronic passive congestion of the liver. In the majority of patients with hepatocellular damage, as in those with extrahepatic obstructive lesions, dye retention occurs only in the presence of hyperbilirubinemia, the degree of retention of dye and pigment being approximately parallel. In some cases, however, this relationship is not maintained. For example, a disproportionately high degree of dye retention may occur in patients with myocardial failure and passive congestion of the liver, as well as in those with hepatic functional impairment complicating thyrotoxicosis, pneumonia and severe anemia. The majority of observers agree that in portal cirrhosis, particularly, dye retention may occur in the absence of hyperbilirubinemia. Under such circumstances this procedure is of value from a diagnostic standpoint.

Investigation of the ability of the liver to eliminate dyes, therefore, is of greatest clinical value in cases of hepatic dysfunction in which hyperbilirubinemia is absent. Among such conditions are certain cases of portal cirrhosis, cholecystitis, cholelithiasis, toxemia of pregnancy, chronic hepatitis, syphilis and various acute infections, such as pneumonia and typhoid fever. The majority of observers state that dye retention occurs in cases of gallbladder disease with frank jaundice but not usually in those with latent or no icterus. In our experience, however, many patients with cholecystitis, with and without cholelithiasis, may show varying degrees of bromsulfalein retention, up to 100 per cent, in the absence of, or with very slight degrees of, hyperbilirubinemia. Such findings indicate

the possibility of an apparent dissociation of two phases of a single function, excretion; that is, the ability of the liver to excrete bilirubin, a normal excretory product, may vary independently of its capacity for eliminating bromsulfalein, a foreign substance.

Rose Bengal Excretion. In normal subjects, when 5-10 cc. of a 1-2 per cent solution of rose bengal are injected intravenously, the concentration of dye in a blood sample withdrawn eight minutes after injection of the dye should be not greater than 50 per cent of that in a sample withdrawn two minutes after injection. Abnormal retention of this dye has the same significance as abnormal retention of bromsulfalein.

SERUM ALKALINE PHOSPHATASE ACTIVITY

The normal range of serum alkaline phosphatase activity is 1.5-4 units (Bodansky method) in adults and 5-14 units in growing children. Variations may occur in this factor in hepatic and biliary tract disease (p. 200).

Serum phosphatase activity is almost invariably increased in the presence of jaundice due to mechanical obstruction of the bile ducts. Values as high as 100 units (Bodansky) have been reported in patients with obstructive jaundice.^{2,3,4,5,7,8,9,10} A notable exception is represented by the report of normal serum phosphatase activity in three infants with congenital atresia of the bile ducts.^{2,4} Some believe that in obstructive jaundice serum phosphatase activity and serum bilirubin concentration increase approximately proportionately until the limit of phosphatase activity is reached (about 40 units).¹⁰ However, this is not invariably the case, occasional instances having been observed of simultaneous high phosphatase values and low serum bilirubin concentrations, and vice versa, in this condition.^{2,6} In cases of partial obstruction of the common bile duct (cholangitis, stone) or in intrahepatic duct obstruction (metastatic malignancy), an increase in serum phosphatase activity may occur with little or no increase in serum bilirubin concentration. A similar phenomenon has been observed in dogs following ligation of single bile ducts.²¹

Increased serum phosphatase activity also occurs in clinical and experimental forms of hepatocellular jaundice.^{2,3,5,12} Values as high as 70 units (Bodansky) have been reported in arsphenamine hepatitis. This, in the case of arsphenamine, is attributed by some observers to intrahepatic bile duct obstruction, since, with the exception of toluylenediamine, other hepatotoxic agents produce relatively slight or moderate increase in phosphatase activity in experimental animals in proportion to the

degree of hyperbilirubinemia. In contrast to obstructive jaundice, values for serum phosphatase activity usually do not parallel the increase in serum bilirubin concentration in hepatocellular jaundice.^{6,10} Whereas high values are encountered occasionally in such cases, in the great majority of instances the serum phosphatase activity is normal or only slightly increased (usually less than 12 units). However, analyses of large groups of cases reveal the fact that no sharp distinction can be made between obstructive and hepatocellular types of jaundice on the basis of the presence or absence of parallelism between the increase in serum phosphatase activity and the rise in serum bilirubin concentration. Furthermore, although a distinctly larger proportion of patients with obstructive than of those with hepatocellular jaundice exhibit very high serum phosphatase values, the relatively wide overlapping of values in the two groups precludes the possibility of differentiating between them on this basis alone. In a study of 300 patients with major disorders of the liver and biliary tract, values above 10 units per 100 cc. were obtained in about 90 per cent of cases of obstruction of the common bile duct, in 18 per cent of cases of hepatocellular jaundice, in about 22 per cent of cases of advanced cirrhosis and in about 50 per cent of cases of liver abscess.⁶

In our opinion, although the estimation of serum alkaline phosphatase activity may give assistance in differential diagnosis in some cases of jaundice, the value of the test in this connection is limited by the following sources of error: (a) there is about 15 per cent overlapping of values in the obstructive and hepatocellular groups in our experience; (b) normal values may be obtained in congenital atresia of the bile ducts; (c) the possibility of extrahepatic lesions, particularly certain skeletal disorders (p. 197), must be considered in interpreting abnormally high values; (d) increases may occur in patients with external bile fistula. However, the combination of high phosphatase (above 20 units) and high serum bilirubin (above 10 mg. per 100 cc.) values supports a diagnosis of obstructive jaundice, and the combination of similar grades of hyperbilirubinemia and phosphatase values below 10 units supports a diagnosis of hepatocellular jaundice.

Serum phosphatase values ranging from normal to over 60 units (Bodansky) per 100 cc. have been observed in patients with portal cirrhosis with and without hyperbilirubinemia.^{2,5} In this condition, no parallelism exists between serum phosphatase activity and serum bilirubin concentration. The same is true of cases of metastatic carcinoma of the liver, in which

condition a rather marked increase in serum phosphatase activity may occur before any increase in serum bilirubin concentration can be demonstrated. Values as high as 35 units per 100 cc. have been reported in such cases in the absence of skeletal metastatic lesions. Increased serum phosphatase activity has also been observed in cases of biliary fistula (clinical and experimental) in which all of the bile was draining externally. Under these circumstances, values of four to ten times normal may be obtained. Correction of the fistula, with the reappearance of bile in the intestine, is usually followed within seven to ten hours by a fall in serum phosphatase activity. No increase has been observed in cases of hemolytic jaundice, a fact which may be of value in differential diagnosis.

Large amounts of phosphatase are excreted daily by the liver in the bile. In view of this fact, it has been suggested that the increase in serum phosphatase activity in jaundice of obstructive and hepatocellular origin may represent a retention phenomenon dependent upon obstruction to the flow of bile, extrahepatic or intrahepatic. This hypothesis would appear to be contradicted by the following observations: (a) the occurrence in portal cirrhosis of marked increase in serum phosphatase activity, at times, with little or no increase in serum bilirubin, and vice versa; (b) normal phosphatase values in cases of congenital atresia of the bile ducts; (c) increased serum phosphatase activity in cases of bile fistula, draining externally. However, the occurrence of increased phosphatase activity with little or no hyperbilirubinemia has been attributed to the fact that phosphatase is apparently not excreted in the urine (except in the cat) whereas bilirubin is readily excreted by the kidneys. The absence of marked increase in phosphatase activity in hepatitis, despite marked hyperbilirubinemia, has been attributed to possible shunting of the enzyme around the damaged hepatic cells.⁶ It is difficult, however, to reconcile the normal values in infants with congenital atresia of the bile ducts with the "retention" theory of the cause of increased serum phosphatase activity in obstructive jaundice. It is believed by some that the liver is an important source of this enzyme, and that the usual absence of marked increase in hepatocellular jaundice is due to diminished formation of phosphatase by the liver. Similarly, these observers believe that in obstructive jaundice, normal or only slightly increased values are obtained if there is associated severe hepatocellular damage, the highest values being obtained when the biliary obstruction is complete and the liver cells are still capable of functioning satisfactorily.¹⁴ However, there is evidence that injured liver cells contain more

phosphatase than normal cells¹² and that serum phosphatase activity in hepatic disease depends, initially at least, on the degree of hepatocellular damage.¹¹ It must be concluded that the mechanism underlying these changes in serum phosphatase activity in hepatic and biliary tract disease is not completely understood.

BLOOD DIASTASE (AMYLASE)

Normal values for blood diastase (Somogyi)¹⁰ range from 60 to 180 units. Values below 60 have been obtained in dogs poisoned with carbon tetrachloride⁶ and in patients with hepatic and biliary tract disease, including acute and chronic cholecystitis, catarrhal jaundice, cirrhosis, hepatitis, acute hepatic necrosis, chronic passive congestion of the liver, fatty liver, and so on.³ Low values in diabetes mellitus, severe burns, thyrotoxicosis, toxemia of pregnancy, acute alcoholism, following surgical operations and in poisoning with chloroform, carbon tetrachloride, arsenic and barbiturates have been attributed to hepatic damage. The cause of the fall in blood diastase in hepatic disease is not known, nor has its relation to the state of hepatic function, as determined by other methods, been ascertained.

High blood diastase levels have been reported in diseases of the liver and biliary tract.^{1,7} It seems probable that in the majority of such cases the increase is due to complicating acute pancreatic disease (p. 497) or to renal functional impairment.³

PLASMA VITAMIN A AND CAROTENE (CAROTENOID) (p. 315)

The normal plasma vitamin A concentration ranges from 100 to 300 I.U. (average 200) per 100 cc. and the plasma carotene from 60 to 260 micrograms (average 145)⁴ (p. 315). Determined by another method,⁶ normal values for vitamin A are 20-43 micrograms and for carotene 100-368 micrograms per 100 cc.⁸ Subnormal values for both vitamin A and carotenoid have been obtained in patients with acute hepatitis and cirrhosis of the liver.^{2,4} The lowest vitamin A values occur in decompensated cirrhosis and are probably related to the development of clinical manifestations of vitamin A deficiency in such cases. This may probably be due to faulty absorption or to fever, in part at least, but it is probably dependent to a large extent upon interference with the intermediary metabolism of vitamin A (inadequate conversion of carotene to vitamin A and inadequate storage of the latter in the liver) (p. 315). The vitamin A content of cirrhotic livers has been shown to be low.⁹

LIVER FUNCTION STUDIES IN DIFFERENTIAL DIAGNOSIS

The most important difficulties encountered in the clinical employment of tests of liver function arise from attempts to interpret functional findings too strictly in terms of disease diagnosis. The fact is too frequently overlooked that tests of function, if they accomplish their purpose, indicate the state of functional activity of an organ and not necessarily the presence or extent of morphologic changes in that organ. This is particularly true of the liver, because of its enormous regenerative capacity and large functional reserve (p. 408). In the case of this, as of other organs, a sharp distinction must be drawn between disease diagnosis and functional diagnosis. However, function tests, used properly, may aid greatly in establishing a disease diagnosis, complementing information obtained by other methods.

The diagnosis of uncomplicated hemolytic jaundice usually offers little difficulty. Apart from the physical and hematological findings, there are characteristically (a) hyperbilirubinemia with a negative direct van den Bergh reaction, (b) little or no increase in direct-reacting serum bilirubin, (c) usually no bilirubin in the urine, (d) increase in urobilinogen in the urine and feces, (e) no bile salts in the urine, (f) hypocholesterolemia and (g) normal findings in other tests of liver function (dye excretion, hippuric acid synthesis, phosphatase activity, galactose tolerance, prothrombin response to vitamin K, cephalin-cholesterol flocculation, etc.). The bilirubin tolerance test may yield abnormal results. In a fairly large proportion of cases of congenital hemolytic jaundice and sickle cell anemia this characteristic picture is complicated by the occurrence of common-duct obstruction by gallstones, with the development of manifestations of obstructive jaundice, which may dominate the clinical picture.

Difficulty is frequently encountered in differentiating between *hepatocellular* and *extrahepatic obstructive* jaundice, and it is chiefly in this connection that attempts are made to utilize function tests as an aid in disease diagnosis. For this purpose it is advisable to group these tests in two categories: (1) tests of "metabolic functions," which depend entirely upon the functional integrity of the liver cells and are uninfluenced by interference with the flow of bile, unless this produces hepatocellular damage; (2) tests of "excretory function," which depend upon maintenance of a free flow of bile as well as upon hepatocellular function. The most important of the "excretory tests" are the serum bilirubin concentration and the dye excretion tests (bromsulfalein; rose bengal). The "metabolic tests" include

the great majority of the other procedures that have been discussed in connection with the estimation of hepatocellular function.

In studying patients with jaundice, the results of the "excretory tests" should be weighed against those of the "metabolic tests." The combination of severe hyperbilirubinemia and marked bromsulfalein retention with normal hippuric acid synthesis, serum phosphatase activity, cholesterol ester concentration, galactose tolerance, prothrombin response to vitamin K, negative cephalin-cholesterol flocculation, etc., indicates serious interference with removal of bromsulfalein and bilirubin from the blood but no disturbance of the other functions investigated. This state of affairs is explicable only on the basis of extrahepatic bile duct obstruction with little or no significant hepatocellular damage. This hypothetical situation is rarely encountered clinically except in very early stages of obstruction of the common duct due to tumor (pancreas, duodenum, bile duct, lymph nodes). Before very long, even in such cases, there develops a variable degree of hepatocellular damage due to increased intraductal pressure or associated factors, and this damage may be reflected in impairment of the "metabolic" functions of the liver. Except in cases of early, partial obstruction, therefore, there is usually some degree of abnormality of one or more of the tests of hepatocellular function ("metabolic tests"). Under such circumstances, evaluation of the relative degree of impairment of the "metabolic" and "excretory" functions may be helpful in differential diagnosis, the latter often being impaired relatively more than the former in primarily obstructive jaundice. Illustrative data in this connection are presented in Tables 14 and 15. However, moderate or severe

TABLE 14
PERCENTAGE OF ABNORMAL FINDINGS IN PATIENTS WITH
JAUNDICE

Condition	Cases	Dye retention	Hippuric acid	Urinary urobilinogen	Cholesterol ester		Prothrombin		Phosphatase	Serum albumin
					Mg	%	Before	After		
Acute hepatitis	270	80	91	85	50	43	78	65	22	30
Cirrhosis	76	68	87	81	59	41	75	52	36	63
Congestive heart failure	50	76	22	64	53	70	-	-	13	70
Stone in common duct	136	100	63	46	31	42	82	38	68	69
Carcinoma pancreas	67	100	43	43	34	64	96	32	78	79

Urinary Urobilinogen: > 1:20

Hippuric Acid: < 2.5 Gm

Cholesterol Ester: < 80 mg.

< 60 per cent

Prothrombin: < 80 per cent

before and after

10 mg Vitamin K i.v.

Phosphatase: > 10 Units

Albumin: < 4 Gm

impairment in both groups of tests, indicating moderate or severe impairment of hepatocellular function and probably extensive hepatocellular damage, does not by any means exclude the possible presence also of extrahepatic bile duct obstruction.

TABLE 15
DEGREE OF IMPAIRMENT OF METABOLIC AS COMPARED TO EXCRETORY
FUNCTIONS OF THE LIVER IN PATIENTS WITH HEPATOCELLULAR
DAMAGE AND EXTRAHEPATIC BILIARY OBSTRUCTION

Condition	Excretory tests	Metabolic tests			
		Normal	Slight	Moderate	Severe
		% of total cases			
Acute hepatitis	Normal	0	0	0	0
	Slight	10	78	12	0
	Moderate	0	65	30	5
	Severe	0	7	46	47
Cirrhosis	Normal	0	0	38	62
	Slight	13	72	15	0
	Moderate	0	29	71	0
	Severe	0	20	67	13
Stone in common duct	Normal	0	0	51	49
	Slight	0	0	0	0
	Moderate	38	34	28	0
	Severe	20	42	18	18
Carcinoma of pancreas	Normal	0	0	58	36
	Slight	0	0	0	0
	Moderate	78	12	10	0
	Severe	34	30	18	18
	Normal	18	24	24	34
	Slight	0	0	0	0
	Moderate	0	0	0	0
	Severe	0	0	0	0

Duodenal intubation and study of the bile are of the utmost importance in the diagnosis of the nature of a biliary tract lesion (pigmented bile duct and gallbladder epithelium, leukocytes, red blood cells and cholesterol and calcium bilirubinate crystals). In a patient with jaundice, failure to obtain bile after prolonged and repeated duodenal intubation and stimulation is very suggestive of complete obstruction of the bile duct. This diagnosis is rendered almost certain by the absence of urobilinogen from the urine and feces (p. 445). In the ultimate analysis, accurate diagnosis in lesions of the liver and bile passages depends primarily upon the clinical history, physical examination, x-ray studies and examination of the bile, supplemented by carefully interpreted functional findings. The last

must not be accorded undue importance in establishing a disease diagnosis. On the other hand, no other findings have much bearing upon the almost equally important matter of evaluation of the adequacy of liver function. The fact must also be borne in mind that there is considerable variation in the sensitivity of the many functions of the liver to noxious influences as well as in the sensitivity of the test procedures employed. For this reason, in the presence of mild functional impairment, significantly abnormal results may be obtained with only one or a few of the tests employed, indicating the necessity of applying as many of the available procedures as possible in all cases. The relative merits and proper interpretation of these procedures have been considered in detail previously.

CHEMICAL EXAMINATION OF BILE^{8,14}

The chemical examination of bile is not often resorted to clinically. However, certain changes which may occur under abnormal conditions are of distinct practical significance. Because of the fact that these changes are usually of a quantitative rather than a qualitative nature, bile obtained by duodenal intubation is seldom satisfactory for such studies because of its admixture with gastric, pancreatic and duodenal secretions. However, even under such circumstances, certain findings, such as the absence of bile salts, are of clinical importance. The most satisfactory and significant studies are those made on bile draining externally, usually through a tube placed in the common bile duct following operation on the bile passages. It is well recognized that, during its sojourn in the gallbladder, bile is concentrated to one-third to one-half of its original volume. The relative proportions of the dissolved solids may also be somewhat altered, particularly in cholecystitis, due to varying degrees of absorption of certain of these constituents from the gallbladder. The extreme rapidity with which water is absorbed by the gallbladder wall is indicated by the fact that the mere passage of bile through the gallbladder may diminish its volume by 50 to 75 per cent. The subsequent discussion will be restricted largely to observations made upon bile obtained by common-duct intubation.

Bile Acids. Bile acids, perhaps the most characteristic constituent of bile, occur in human bile chiefly as salts of the cholic and deoxycholic acid series and their derivatives. Of great interest and importance is the fact that these substances are derivatives of cholane, and that the cholane ring system forms the nucleus of an apparently unconnected series of physiologically highly active substances, including vitamin D, cholesterol,

certain estrogenic hormones, carcinogenic hydrocarbons, cardiac glucosides and certain toad poisons.^{2,3,6} The identity of the fundamental ring systems of cholesterol and the bile acids suggests an intimate metabolic relationship of these two substances. Bile acids are specific products of hepatic-cell activity and occur in human bile chiefly as conjugate amino acids, being combined with glycine and taurine. Practically all if not all of the bile acids are in the form of salts of one of the alkalis, chiefly sodium. Glycocholic acid predominates over taurocholic acid under normal conditions in human bile. Variations in the ratio between these two compounds are explained by Sobotka as follows: Cholic acid has a greater affinity for taurine than for glycine and is therefore first conjugated with all the available taurine. Only an excess of cholic acid over taurine will combine with glycine. The ratio, glyco: taurocholic acid is thus regulated by the amount of both amino acids and the amount of free bile acid available. When bile acid is present in low concentration, as is the case immediately following release of biliary obstruction of long duration, the chief combination is with taurine. Later, as the amount of bile acid returns to normal, more than half is available for combination with glycine. Under normal conditions, 85-90 per cent of the bile acids entering the intestine is reabsorbed in the portal circulation and by the lymphatics, only 10-15 per cent escaping in the feces. In patients with diarrhea the loss in the feces may be greater.

A decrease in the bile acid content of the bile has been observed in clinical states of extensive hepatic damage, experimentally following the administration of hepatotoxic agents and following prolonged periods of total bile stasis. Ravdin and his associates found them to be absent from the bile of all patients studied in whom complete biliary obstruction had been present for a week or longer. The interval between the time of release of obstruction and the reappearance of bile acids in the hepatic bile was apparently roughly proportional to the degree and duration of obstruction and to the degree of associated cholangitis, being in some instances as long as twenty-seven days. This observation has a distinct bearing on prognosis following operation in such cases, since it indicates the extent of impairment of liver function. However, it has been suggested that these findings may be due to the prevalence of bile acids which do not respond to the Pettenkofer reaction during the first days of resumption of chologenesis (p. 454), as the colorimetric test yields figures which lag behind those obtained by gravimetric methods in some cases.¹ It is interesting also that Riegel has found a decrease in the bile acid concentration of gallbladder

bile in normal pregnant women, but no studies are available regarding its concentration in the liver bile during pregnancy. Under normal conditions, the bile acid concentration of human liver bile has been found to range from 200 to 1800 mg. per 100 cc.

Lipids.^{2,3,6} Normal bile contains fatty acids, neutral fat, phosphatides and cholesterol, the last being by far the most important of these lipids because of its relation to the formation of gallstones. The cholesterol concentration of normal human liver bile ranges from about 20 to 200 mg. per 100 cc., usually being well below its concentration in the blood plasma. Whereas 60-80 per cent of the plasma cholesterol is in the form of esters, in the great majority of cases studied all of the bile cholesterol was in the free state. Indeed, it seems questionable whether cholesterol esters are present normally in liver bile unless it is contaminated with blood.

Studies of the effect of diet upon the cholesterol content of liver bile has been largely confined, for obvious reasons, to experimental animals. Caution must be exercised in applying such observations to human beings, owing to fundamental differences in the physiology of the biliary system in different species. However, certain of these observations may perhaps be applied clinically. It has been found that although the total output of cholesterol may possibly be influenced by dietary factors, its concentration in the bile remains practically unaltered even after feeding enormous amounts. Moreover, there appears to be no consistent relationship between the level of plasma cholesterol and its concentration in the bile. It is possible to raise the blood cholesterol without a corresponding increase in bile cholesterol and also to increase the cholesterol elimination in the bile without any alteration in the blood cholesterol concentration. An increase in the cholesterol content of both hepatic and gallbladder bile occurs in the late months of pregnancy and persists until shortly after birth of the child.

The lack of relationship between plasma and bile cholesterol is emphasized by the fact that in some cases of the nephrotic syndrome and diabetes mellitus with extremely high plasma cholesterol values, the bile cholesterol concentration may be low, while in some cases of pernicious anemia, with a relatively low plasma cholesterol concentration, the level in the bile may actually exceed that in the blood. The cholesterol content of liver bile is diminished in the presence of severe hepatic disease as well as during and following the relief of total bile stasis.¹¹ Values of less than 10 mg. per 100 cc. of bile have been observed under certain circumstances, the degree of diminution and the

interval elapsing before a return to normal being dependent apparently upon the extent of liver damage.

Inorganic Elements. The molar concentration of inorganic ions in hepatic bile is approximately the same as in blood serum. The one exception is magnesium which, according to some observers, is present only in traces in liver bile (0.5 mg. per 100 cc. in the dog). From a clinical standpoint, interest is centered particularly in changes which have been observed in the chloride concentration following release of biliary obstruction. It has been found that the chloride concentration of hepatic bile is usually higher than that of the blood plasma immediately after release of the obstruction. Persistence of this high level or a continued increase has been found to be of serious prognostic significance, while recovery is accompanied by a return of the bile chloride concentration to normal levels. These changes are consistent enough to be of some clinical significance in prognosis. The calcium content of hepatic bile immediately after release of biliary obstruction appears to be somewhat lower than normal.

Miscellaneous Constituents. Bile normally contains mucoprotein, ammonia, urea, purine derivatives and amino acids. Leucine and tyrosine appear in the bile in cases of severe hepatic necrosis. Relatively small amounts of glucose are also present (less than 70 mg. per 100 cc.) in the fasting state, increasing after the ingestion of foods and in abnormal hyperglycemic states. The usual absence of glucose from gallbladder bile is attributed to rapid glycolysis in that viscus. Only traces of amylase and no lipolytic or proteolytic enzymes are usually present. The only enzyme of significance present in abundance in hepatic bile is phosphatase (p. 462). Bile also constitutes an excretory medium for many substances, including lead, copper, bismuth, arsenic, mercury, iron, iodine, sulfonamides, salicylates, etc.

It has been found that large amounts of exogenous and endogenous estrogens are excreted in the bile.⁴ There is evidence that these undergo an enterohepatic circulation similar to that of bile acids. Vitamin D is excreted in the bile⁷ and androgens have also been found.¹⁰ It seems probable that the bile may be an important medium of excretion of other steroid compounds.

Bile Pigment. Bilirubin and biliverdin are the most important pigments of normal human bile, the latter being an oxidation product of the former. The pigment of freshly secreted human bile consists practically entirely of bilirubin. Its concentration varies considerably from time to time, the range of values usually quoted being 2.3 to 18 mg. per 100 cc.¹³ However,

values of over 400 mg. per 100 cc. have been reported. It has been found that in biliary obstruction there is a diminution in the amount of pigment in the bile, this change being masked at times by the concentrating action of the gallbladder.^{1,9} With obstruction of relatively brief duration there is a rapid increase in both concentration and total output of bile pigment during the greater part of the first week of decompression, with a subsequent decrease to approximately normal levels. If obstruction has been present long enough to produce permanent hepatic damage, or in the presence of hepatic dysfunction due to other cause, there is a decrease in both the concentration and the total output of bile pigment, frequently with the excretion of relatively large quantities of pale fluid with a low total solid concentration.

White Bile. When any portion of a damaged extrahepatic biliary conducting system becomes obstructed, the static bile is gradually diluted by the mucoid secretion of the duct epithelium and eventually becomes colorless, the bile pigment being either absorbed or transformed into a colorless substance.⁹ This "white bile" is important because of its great prognostic significance when found in the common bile duct. The mechanism of its production has been the subject of considerable investigation. Recent studies indicate that "white bile" obtained from the gallbladder contains chloride and, as a rule, calcium at approximately the blood serum levels, the calcium being somewhat more variable than the chloride.¹² The cholesterol concentration is very low and bile acids are absent, bile pigment being present in extremely small amounts or being entirely absent. The consensus of opinion is that "white bile" can be formed only if the hepatic parenchyma is no longer functioning normally and that it is largely, if not entirely, a product of the activity of the mucosal cells of the biliary conducting system.

Gallstones. Gallstones, in man, usually consist of cholesterol, either alone or with admixtures of calcium bilirubinate, carbonate or phosphate. Pure pigment stones are found only occasionally. Gallstones may form whenever the concentration of cholesterol in the bile exceeds its saturation point. This may happen because of an increase in the concentration of cholesterol or a decrease in its solubility in the bile. Recent studies emphasize the significance of the quantitative relationship between bile salts and cholesterol in the bile in preserving the state of solution of the latter under normal conditions and in determining precipitation in the presence of disease of the bile passages.³ According to Sobotka, decreased solubility of cholesterol in the bile may be due to the following causes: (a) during stasis in

interval elapsing before a return to normal being dependent apparently upon the extent of liver damage.

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the gallbladder, the pH of bile is lowered and the faculty of bile acids for "complex" formation is diminished, with a diminution also in their dissolving power; (b) resorption of water by the gallbladder mucosa raises the concentration of all constituents of the bile, which brings most of the bile components to the limit of their saturation and leads to precipitation: (c) bile acids may be reabsorbed through an inflamed gallbladder mucosa so rapidly that the bile acid:cholesterol ratio falls to the point where cholesterol is precipitated. The disturbance in this ratio may be aggravated by the fact that the inflamed gallbladder mucosa may add relatively large quantities of cholesterol to the contained bile. This may be due either to active excretion or to desquamation of cholesterol-containing mucosal cells.

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Chapter XX

Chemical Investigation of Gastric Function^{1,5,11}

CHEMICAL examination of the gastric contents may yield valuable information regarding both the secretory and the motor activities of the stomach, and may reveal the presence of abnormal substances indicative of pathologic conditions. Microscopical study constitutes an essential part of the examination of the gastric contents but must necessarily be omitted from a discussion of the chemical aspects of functional diagnosis. In order to obtain complete evidence regarding the state of gastric functional activity, the contents of the stomach must be examined both during the interdigestive period (fasting stomach) and during the period of digestion, or stimulation.

The most important specific secretory products of gastric activity are (1) hydrochloric acid, (2) enzymes (pepsin, rennin and a weak lipase) and (3) mucus. Gastric juice contains also amino acids, histamine and other amines, urea, ammonia and neutral inorganic salts, such as chlorides. The true gastric glands, *i.e.*, those which secrete HCl and enzymes, occur in the body and fundus, while the glands of the pylorus and cardia secrete mucus but probably no HCl and little or no pepsin. There are three types of cell in the true gastric glands: (a) parietal or border cells, which secrete HCl at a rather constant concentration of 0.154–0.2 normal; (b) chief cells of the body of the glands, which secrete pepsin; (c) chief cells of the neck of the glands, which secrete mucus ("dissolved mucus").

Gastric secretion is commonly described as consisting of three phases, (1) a psychic or cephalic phase, (2) a gastric phase and (3) an intestinal phase.

Psychic or Cephalic Phase. This is due to a nervous mechanism mediated through the vagus and perhaps also the splanchnics to a certain extent. It is evoked by pleasurable sensations accompanying the thought, sight, smell and taste of palatable food, and has been elicited by hypnotic suggestion. Inhibition of secretion may result from psychic influences such as worry and anxiety, and the sight or smell of disagreeable food. Psychic stimuli (vagus) produce a secretion of very high pepsin and relatively or absolutely low HCl content.

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Examination of Bile

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istics are of importance from the standpoint of functional diagnosis.

Amount. The quantity of material normally found in the fasting stomach varies from 20 to 100 cc., averaging about 50 cc. An increase in the volume of the gastric residuum may be due to hypersecretion, retention or regurgitation from the duodenum. These conditions may be differentiated on the basis of other observations such as the presence of food particles in retention, the presence of abnormal quantities of bile and duodenal enzymes in regurgitation, and the absence of these factors in true hypersecretion, in which condition the gastric contents, apart from their increased volume and perhaps increased acidity, may be essentially normal.

Color. Freshly secreted gastric juice is colorless. However, in about 55 per cent of normal individuals the gastric residuum is either yellow or green, due to regurgitation of bile from the duodenum, which occurs in about 25 per cent of normal individuals, or to the presence of molds or the *Cryptococcus salmonicus*. Increased quantities of bile in the stomach, when not due to retching incident to the passage of the tube, result from intestinal obstruction or ileal stasis. A bright or dark red, brown or black color in the residuum is usually indicative of the presence of blood (see below).

Consistency. The normal gastric residuum is rather fluid in consistency, containing no solid food particles and only a small quantity of ropy mucus which may be derived from the nasopharynx. The presence of an increased quantity of sediment is usually indicative of retention, and increased quantities of mucus are found in catarrhal inflammations of the stomach.

Blood. The benzidine test is the one most commonly employed for the detection of blood in the gastric contents. A trace of bright red, aerated blood in specimens extracted through the ordinary metal-tipped tube is most commonly due to accidental trauma to the gastric mucous membrane. The incidence of accidental blood may be diminished by the use of a paraffin or rubber-coated tip. Pathologically, the gross appearance of specimens containing blood depends upon the extent of the hemorrhage, the length of time the blood has remained in the stomach and the degree of acidity of the gastric contents. In the presence of hydrochloric acid the red cells are hemolyzed, the hemoglobin being converted into acid hematin which is dark brown in color. Blood which has resided in the stomach for relatively long periods of time is therefore usually dark and well mixed with the gastric contents. If coagulation has occurred, as is frequently the case in carcinoma, the clot may be par-

Gastric Phase. Chemical stimulation of the gastric mucosa, particularly in the pylorus, causes the release of a substance (gastric hormone) which, transmitted by the blood stream, acts as a gastric secretagogue. There is evidence that a local nervous mechanism (vagus terminals in the stomach wall) is involved in the production or liberation of this hormone. It is not entirely clear at present whether this factor is simply histamine (p. 482); the latter produces a secretion of maximal acidity but very low in pepsin, differing in this respect from normal gastric juice.

Intestinal Phase. Certain products of gastric digestion, when they enter the duodenum, act as chemical excitants to gastric secretion. This is true also of water, meat extracts, albumoses, peptones, saponin, soaps and magnesium sulfate. Although a substance ("gastric secretin") which stimulates gastric secretion has been extracted from the intestinal mucosa, it is generally believed that the intestinal phase is due to secretagogues in the food, absorbed from the intestine, the vagus terminals in the wall of the stomach or intestine being involved in the mechanism.

The intestinal phase of gastric secretion is inhibited by fat (before absorption), the acidity, volume and especially peptic activity being lowered. A similar effect is produced by injection of extracts of intestinal mucosa or urine; the factors responsible for this effect have been termed enterogastrone and urogastrone, respectively.

Other Factors. Insulin (hypoglycemia) stimulates gastric secretion through the vagus mechanism, the volume, acidity and peptic activity of the gastric juice being increased. Mécboyl, pilocarpine and nicotine increase the volume considerably and the acidity somewhat. Alcohol is a powerful stimulant (acidity and volume), while acids and atropine are secretory depressants.

Different foodstuffs influence the secretion in different ways. For example, meat produces a juice of high acid and moderate pepsin content, bread a juice of low acid and high pepsin content, and milk a juice of moderate acid and low pepsin content. Fat depresses peptic activity relatively more than acidity or volume. It has been suggested that the various secreting cells (chief, parietal, mucous) are stimulated in varying degree by the same as well as by different stimuli. It is more likely, however, that various influences involved (psychic, nervous, chemical) stimulate or inhibit each set of secretory elements separately.¹²

GASTRIC RESIDUUM

Valuable information may be obtained by the examination of the stomach contents during the interdigestive period (gastric residuum). The following chemical and gross physical character-

free hydrochloric acid may be normally absent and the total acidity extremely low. Free HCl is practically never found when the total acidity is below 10. The absence of both free HCl and total acidity, particularly if pepsin and rennin are also lacking, may however be of significance. The interpretation of hypoacidity will be considered in the discussion of fractional gastric analysis. Free HCl values above 30 or total acid values above 50 should be regarded as abnormally high, constituting a state of hyperacidity, the significance of which will be considered below.

FRACTIONAL GASTRIC ANALYSIS

The procedure of fractional gastric analysis constitutes a means of studying the manner in which the stomach responds to stimulation. The stimulation may be artificial, as represented by the injection of histamine or the administration of alcohol, or it may be natural in the form of various foodstuffs introduced into the stomach. Each form of stimulation possesses advantages under certain circumstances, the injection of histamine being of particular value in obtaining a maximum secretory response and in establishing a diagnosis of true achlorhydria, the alcohol meal being of value in cases in which the quantitative determination of the volume of gastric secretion is desirable. The ordinary foodstuff meals possess the advantage of yielding information concerning gastric digestion and the motor activity of the stomach.

Ewald Test Meal. This consists of either one roll or two slices of wheat bread or toast weighing approximately 35 Gm. (no butter) and eight ounces of water or weak tea (no sugar or cream). A shredded wheat biscuit or soda biscuits may be substituted for the bread. This combination is the one most commonly employed in routine practice. It serves as a bland stimulant of gastric secretory activity and should be replaced by a more stimulating meal in cases of hypoacidity or suspected achylia.

Boas Meal. This consists of one tablespoonful of oatmeal boiled in 800 cc. of water until the volume is reduced to 400 cc. This meal contains no lactic acid or yeasts which may be present in bread and is therefore particularly useful in cases in which the presence of lactic acid is suspected.

Riegel Meal. This consists of about 200 cc. of beef broth, 150-200 Gm. of broiled beef steak and about 100 Gm. of mashed potatoes. This combination is useful where a maximum secretory response is desired and is therefore particularly indicated in cases of hypochlorhydria or suspected achylia. The acid values

tially disintegrated, having the so-called characteristic "coffee-grounds" appearance. The presence of blood in the gastric residuum may be due to such lesions as carcinoma of the stomach, portal cirrhosis, chronic passive congestion of the stomach, peptic ulcer, gastric lues, acute gastritis and hemorrhagic blood dyscrasias, including purpura hemorrhagica, acute leukemia, agranulocytosis, aplastic anemia, and so on. It must be remembered that blood derived from the gums and from nasopharyngeal, laryngeal, tracheobronchial and pulmonary lesions may be swallowed.

Organic Acids. Lactic, butyric and other fatty acids may be found in the gastric contents. Some observers believe that lactic acid is secreted by the gastric mucosa in carcinoma of the stomach; however, the consensus of opinion is that organic acids result from stagnation of the gastric contents with consequent bacterial action and fermentation. Since these processes are inhibited by high free acidity, lactic acid is most commonly found in association with gastric retention and hypochlorhydria, the combination of the three being rather significant of carcinoma of the stomach.

Enzymes. The normal gastric residuum contains pepsin and rennin. Trypsin is frequently found to be present as a result of regurgitation from the duodenum. The absence of pepsin and rennin, occurring in conjunction with achlorhydria, constitutes true achylia, which is encountered typically in pernicious anemia and in subacute combined degeneration of the spinal cord. These enzymes are practically never absent if free hydrochloric acid is present in the gastric secretion.

Free and Total Acidity. Gastric acidity is usually expressed in terms of the number of cubic centimeters of tenth-normal sodium hydroxide required to neutralize 100 cc. of gastric contents. The free HCl content of the residuum normally ranges from 0 to 30, with an average value of 18.5 (cc. N/10 NaOH to neutralize 100 cc.). This is at times expressed in terms of grams of hydrochloric acid per 100 cc. of gastric contents, ranging from 0 to 0.1095 Gm., with an average of 0.0675 Gm. The total acidity includes acidity due to free hydrochloric acid, hydrochloric acid combined with protein, acid salts (phosphates and carbonates) and organic acids such as lactic and butyric acid (abnormal). The total acidity of the normal residuum ranges from 10 to 50 (cc. N/10 NaOH to neutralize 100 cc.), averaging 30. In terms of grams HCl per 100 cc., the total acidity ranges from 0.0365 to 0.1825 Gm., with an average value of 0.1095 Gm.

A condition of hyp acidity cannot be diagnosed with justification by the examination of the gastric residuum since

free HCl, if not present in the gastric residuum, usually appears in the first sample. It rises gradually, reaching a maximum of 40-60 in from sixty to seventy-five minutes, then falling gradually to 10 to 15 in two to two and one-half hours. Free HCl is almost never present with total acidity values below 10 and is practically never absent if this value is above 13.5. The curve of total acidity parallels that of free HCl, usually reaching a maximum of 60 to 70.

(2) **Hypersecretory** (33 per cent). Free HCl is usually present in the gastric residuum, rising rather rapidly to reach a

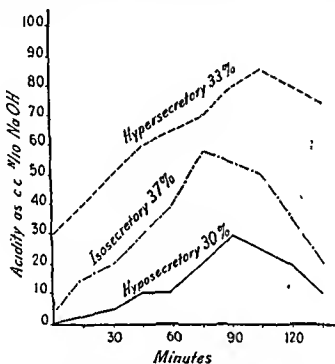


Fig. 17.—Normal free HCl curves

maximum of 70 to 90 in about two hours, then declining rather slowly, gastric secretory activity continuing in many cases for some hours after all food has left the stomach.

(3) **Hyposecretory** (30 per cent). Free HCl is absent in the gastric residuum and rises very slowly to a maximum of 30 to 40.

It is apparent that normal individuals exhibit an extremely wide variation in the response to gastric stimulation. It is believed, however, that the response of each individual remains quite constant under the same conditions of stimulation. Values tend to be somewhat lower in women than in men and lower in children than in adults (after twenty years). The acidity tends to fall after thirty years of age in men and after fifty years in women.

The normal curves of acidity reach a maximum in one to two hours and then begin to fall. The normal decline is probably due

are sometimes considerably higher than those obtained by means of the Ewald meal.

Ehrmann Alcohol Meal. This test meal, modified and recommended by Bloomfield and Kiefer, consists of 50 cc. of 7 per cent alcohol. As stated by these observers, it possesses the advantages of readiness of administration, ease of withdrawal of samples, ease of examination of specimens, and the possibility of a more exact quantitative determination of the volume of gastric secretion. On the other hand, it affords little information regarding the motor activity of the stomach.

Histamine Stimulation. Histamine is a powerful gastric secretory stimulant, producing a juice of maximal acidity and relatively low pepsin content. It is injected subcutaneously in a dosage of 0.01 mg. per kilogram of body weight. Some employ a standard total dose of 0.5 mg. and others 0.25 mg. With histamine stimulation, the free acidity rises rather promptly, reaching a maximum usually in forty to sixty minutes and then declining. The volume of gastric juice varies correspondingly. The maximum response in normal subjects, excluding those with achlorhydria, may range from 30-160 units (30-160 millimols HCl or 0.1-0.6 per cent HCl). Because it produces a maximal acid response, this procedure must be employed before a diagnosis of achlorhydria can be established with certainty.

A double histamine test has been proposed for demonstrating the capacity of the stomach for maintaining a maximal secretion over prolonged periods of time.⁹ The second injection is given sixty to ninety minutes after the first, when the initial curve is beginning to fall. In normal subjects a second rise and fall occur, similar to the first curve in shape and magnitude. In subjects with hypersecretion (e.g., peptic ulcer), the peak of acidity reached after the first stimulation is maintained at a relatively constant level during the double test period.

After the withdrawal of the gastric residuum and the administration of the test meal, 5 cc. of gastric contents are aspirated every fifteen to twenty minutes for a total of two to three hours, or, better, until the stomach is empty. Each sample is routinely examined for the substances considered in dealing with the gastric residuum. In some cases, in addition, the total chloride content is determined.

NORMAL RESPONSE

According to Rehfuess, three types of normal response may be obtained (Ewald Meal).

(1) **Isosecretory** (37 per cent). In this group of individuals

hydria, associated with none of the recognized causes for the absence of free HCl, occurring probably on the basis of some constitutional defect, and showing a definite hereditary and familial tendency. Many of these are instances of true achylia,

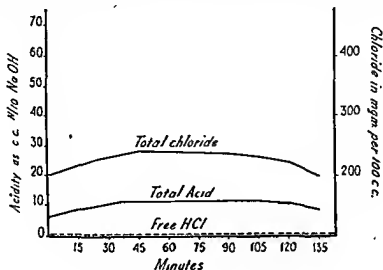


Fig. 18.—True achlorhydria.

gastric enzymes being also absent. This condition is of particular interest because of the fact that such individuals appear to be predisposed to the development of pernicious anemia.

Hypoacidity. The term "hypoacidity" is applied to acid

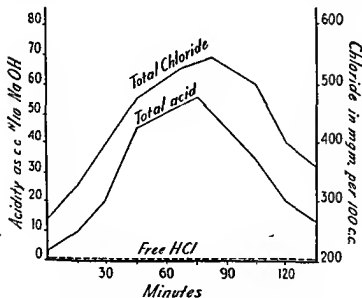


Fig. 19.—False achlorhydria.

curves which are lower than that known to be normal for any individual or, in any case, lower than that characterized by the hyposecretory type of normal response. Hypoacidity is commonly observed in carcinoma of the stomach, chronic gastritis,

chiefly to (a) reduction in the volume of secretion of the glands of the fundus and body and (b) evacuation of the stomach. Some believe that the gastric acidity is normally reduced by regurgitation of alkaline fluid (pancreatic juice) from the duodenum and by saliva, food products and the alkaline secretion of the pyloric mucosa. It has been shown, however, that although these phenomena do occur, they are not necessary for the production of the normal fall in gastric acidity. According to some, back-diffusion of HCl across the gastric mucosa contributes to the drop in acidity.⁸

ABNORMAL RESPONSE

From the standpoint of acidity, three main types of response may be obtained under pathologic conditions. The following classification is adapted largely from the work of Rehfuess.

Achlorhydria. The term "achlorhydria" is applied to the absence of free hydrochloric acid in all samples obtained during the digestive period. In some cases the total acidity may rise to 20 to 30, the curve being practically horizontal. The term "true achlorhydria" is applied to the absence of free HCl from freshly secreted gastric juice. The term "false achlorhydria" is applied to the absence of free HCl from the withdrawn samples due to the fact that it has been combined with and neutralized by the ingested material, duodenal regurgitation, saliva or mucous secretion of the stomach. The two conditions may be differentiated by the determination of the total chloride concentration of the gastric contents, which affords a more accurate index of the actual amount of secreted hydrochloric acid, being normal in false achlorhydria and low in true achlorhydria. True achlorhydria is most commonly observed in pernicious anemia, gastric carcinoma, chronic gastritis, gastric neuroses, oral sepsis, subacute combined degeneration of the cord and severe anemias. Achlorhydria is also encountered occasionally in patients with hyperthyroidism, adrenal insufficiency, diabetes mellitus, pulmonary tuberculosis and generalized arteriosclerosis. It is present in less than 6 per cent of healthy young adults (twenty to forty years), but increases with advancing age, having been found in 20 to 30 per cent of patients without gastric disease between the ages of sixty and seventy years.^{7,13}

The existence of achlorhydria can be determined with certainty only by histamine stimulation. This produces a maximal acid response, ranging from 30 to 160 clinical units in normal subjects (excluding achlorhydria), with minute volumes of gastric juice of 15-60 cc. (average 30 cc.).^{2,6,10}

There is in some individuals a condition of primary achlor-

hydria, associated with none of the recognized causes for the absence of free HCl, occurring probably on the basis of some constitutional defect, and showing a definite hereditary and familial tendency. Many of these are instances of true achylia,

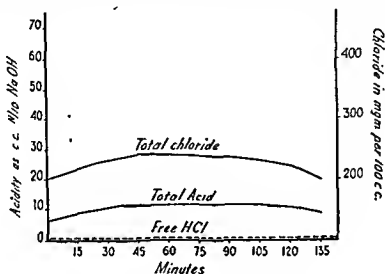


Fig. 18.—True achlorhydria.

gastric enzymes being also absent. This condition is of particular interest because of the fact that such individuals appear to be predisposed to the development of pernicious anemia.

Hypoacidity. The term "hypoacidity" is applied to acid

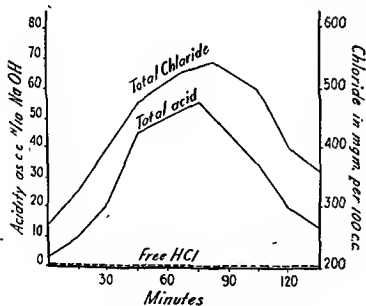


Fig. 19.—False achlorhydria.

curves which are lower than that known to be normal for any individual or, in any case, lower than that characterized by the hyposecretory type of normal response. Hypoacidity is commonly observed in carcinoma of the stomach, chronic gastritis,

chronic constipation, chronic appendicitis, gastric and other neuroses, mucous colitis, secondary anemia, chronic debilitating disease, particularly tuberculosis, in hyperthyroidism, some cases of gastric, and rarely, duodenal ulcer and in about 20 per cent of normal individuals, some of whom perhaps belong more properly in the group of neurotics. Subnormal secretion of free HCl and pepsin has been observed in about 75 percent of normal pregnant women. This observation may be of significance in relation to the development of both iron deficiency anemia (defective iron absorption) and the so-called "pernicious anemia of pregnancy."

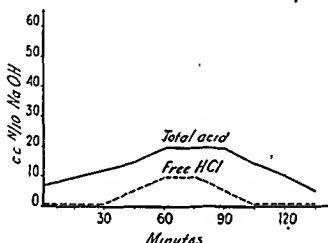


Fig. 20.—Hypochlorhydria.

Hyperacidity. Certain factors must always be kept in mind in interpreting acid curves above the high normal level. It must be remembered that acid values obtained following the ingestion of meat meals are higher than those obtained by the use of the Ewald or Boas diet. A small proportion of normal individuals exhibit high acid values (100–120 total acidity) during the digestive period. More emphasis must be placed upon the interdigestive acidity, the type of acid curve and the rate of gastric evacuation than upon the actual height to which the acidity rises during the digestive stage. Hyperacidity does not imply that the gastric glands are secreting a juice of abnormally high acidity. There is no evidence that the normal maximum of 0.5–0.6 per cent HCl (160 clinical units) is ever exceeded. Clinical hyperacidity is due to one or more of the following factors: (a) secretion of an abnormally large volume of gastric juice (hypersecretion), (b) failure of the volume of secretion to diminish during the second hour and (c) delayed gastric evacuation. Rehfuess has classified the various types of hyperacid curves as follows:

(a) *Larval Hyperacidity.* There is a sharp rise in acidity

within the first hour of digestion, the remainder of the curve being normal and the residual acidity being within normal limits. Larval hyperacidity may be due to excessive gastric secretion. It is commonly observed in certain gastric neuroses and occasionally in duodenal ulcer. Little practical significance can be attached to this type of acid reaction.

(b) *Digestive Hyperacidity*. This type of reaction resembles the normal hypersecretory curve but may be greatly exagger-

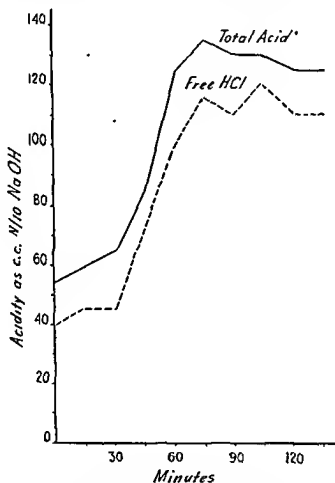


Fig. 21.—Hyperchlorhydria.

ated and prolonged. The maximum acidity coincides with the height of the digestive period. Digestive hyperacidity is observed in cases of gastric neurosis (vagotonia) and in some patients with duodenal and gastric ulcer and cholecystitis.

(c) *Postdigestive Hyperacidity*. The gastric acidity increases steadily throughout the entire digestive period, reaching a maximum in the latter portion of the cycle and being maintained into the interdigestive period. This type of curve is rather characteristic of hyperirritability and pylorospasm but is sometimes due to hypersecretion. It is found in from 70 to 80 per cent of patients with duodenal ulcer, in many individuals with

cholecystitis, in cases of chronic appendicitis and chronic constipation and occasionally in gastric ulcer.

(d) *Interdigestive Hyperacidity*. This type of response may be observed as an independent phenomenon or merely as a prolongation of the postdigestive type of hyperacidity. It is observed most frequently in patients with duodenal ulcer or with chronic cholecystitis and duodenal adhesions but may also be obtained in individuals with gastric neurosis (vagotonia) and in normal individuals who use tobacco to excess.

(e) *Plateau Curve*. In this type of response, which is merely an exaggeration of interdigestive hyperacidity, the entire interdigestive portion of the curve is obliterated. The acidity may fall immediately following the ingestion of a meal, due to dilution of the gastric juice. This preliminary fall is rapidly followed by a sharp rise, the acidity being maintained at a high level for several hours. A plateau curve is characteristic of pyloric stenosis of an advanced degree associated with active gastric secretory activity (stenosing peptic ulcer).

PEPTIC ACTIVITY

The normal gastric secretion contains pepsin and rennin. Trypsin may also be present in the gastric contents as a result of regurgitation from the duodenum. Pepsin is never absent from the gastric juice if free hydrochloric acid is present. However, in the absence of free hydrochloric acid, the determination of pepsin is of extreme importance in distinguishing between achlorhydria and achylia.

Achylia is the term applied to the absence of both free hydrochloric acid and gastric enzymes, the total acidity ranging from 0 to 15. True achylia may occur in pernicious anemia, subacute combined degeneration of the cord, severe secondary anemia and following gastro-enteritis in children. Occasionally, free hydrochloric acid and pepsin may be absent from specimens withdrawn following the administration of a test meal, but will be found following the production of maximum stimulation by means of histamine injected subcutaneously. The latter procedure is of great value in distinguishing between true and false achylia. True achylia is perhaps the most significant feature of pernicious anemia from the standpoint of laboratory diagnosis, it being doubtful whether such a diagnosis is justifiable in the presence of either free hydrochloric acid or gastric enzymes.

EVACUATION TIME

Following the ingestion of an Ewald or Boas meal the average normal stomach empties in approximately two hours. However,

in a certain proportion of normal individuals, particularly those with the hyposecretory type of acid curve, the stomach may empty more rapidly (in from one to one and one-fourth hours). In another group, particularly those manifesting the hypersecretory type of curve, gastric emptying is delayed in some cases for as long as three hours. The emptying time of the stomach is indicated by the disappearance of food residue in the aspirated samples, by the failure of response to the iodine test for starch, or, in the absence of free hydrochloric acid, by a negative test for sugar which results from the digestion of starch by ptyalin in swallowed saliva.

TOTAL CHLORIDE

Several factors may operate under normal and abnormal circumstances to modify the state in which HCl exists in the stomach. A portion may remain as secreted in the free state, a portion may combine with proteins present in the stomach and a variable portion may exist in inorganic combination. In the event of excessive regurgitation of duodenal contents into the stomach or excessive alkaline secretion of the pyloric glands, a large proportion of the secreted hydrochloric acid may be neutralized, and under such circumstances neither the curve of free HCl or that of total acidity can be regarded as indicating the true state of gastric secretory activity. Bolton and Goodhart have emphasized the fact that the curve of total chloride is more nearly representative of the true state of gastric secretory activity since it includes, in addition to free hydrochloric acid and HCl combined with protein, salts of hydrochloric acid which are neutral in reaction. It has frequently been observed that the total chloride curve may continue to rise for a variable period after the acid curve has begun to fall, indicating that HCl is still being secreted but is being neutralized. The determination of total chloride in the gastric content is particularly valuable in the differentiation between true and false achlorhydria; in true achlorhydria no free HCl appears in the gastric content during the digestive cycle and the curve of total chloride is correspondingly low; in false achlorhydria a normal amount of HCl may be secreted but is not present in the free state in the gastric content because of excessive neutralization, and under such circumstances the curve of total chloride may be essentially normal (see Figs. 18, 19).

The total chloride content of the fasting juice of normal subjects ranges from 160 to 550 mg. per 100 cc., or 45 to 155 milliequivalents per liter. With histamine stimulation it may reach a maximum of about 600 mg. per 100 cc., or about 172 m. Eq.

per liter. The total chlorides vary within narrower limits than the free acidity.

BILE

Bile pigment may be observed in the gastric residuum in from 25 to 55 per cent of normal individuals. It is particularly frequently observed if violent retching has occurred during the passage of the tube. It is believed by some observers that regurgitation from the duodenum constitutes one of the normal mechanisms for the neutralization of gastric acidity and, as such, occurs particularly in the later stages of the digestive cycle but may occur to a certain extent throughout the entire period. However, the presence of large amounts of bile in all of the specimens extracted during the digestive phase of gastric activity should be considered abnormal, being usually dependent upon relatively increased intraduodenal tension with a patent pylorus.

VOMITUS

Vomit should be subjected to the same routine examination as the gastric residuum. Vomit obtained during the digestive period should partake of the characteristics of test specimens removed following the ingestion of a stimulating meal, that obtained during the interdigestive phase constituting essentially the fasting contents of the stomach. The observations made regarding the significance of various findings in the aspirated gastric contents apply equally to the vomitus.

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Chapter XXI

Pancreatic Function

THE metabolic significance of the pancreas lies in its internal and external secretions. The internal secretion, insulin, is produced in the islands of Langerhans, absorbed directly into the blood and plays an important part in the metabolism of carbohydrates, as is indicated in the consideration of carbohydrate metabolism (p. 5) and diabetes mellitus (p. 332). The external secretion, produced by the acinous cells of the pancreas, is strongly alkaline in reaction due to the presence of bicarbonate, and contains a small amount of coagulable protein and inorganic constituents, the most important constituents, however, from a functional standpoint, being three enzymes or their zymogens, namely, trypsin, a proteolytic enzyme, pancreatic diastase (amylase), an amylolytic enzyme, and lipase, a lipolytic enzyme. The determination of pancreatic functional efficiency will be discussed from the standpoint of its external secretion, since its internal secretory function is affected in a relatively small proportion of cases of pancreatic disease (about 35 per cent), and is considered in detail elsewhere (p. 332).

Pancreatic acinar secretion is under hormonal and nervous control. *Secretin* is the term applied to a pancreatic secretagogue obtained by extraction of the upper intestinal mucosa. Entering the blood stream, it exerts a rather specific effect upon the acinal cells, producing an increased volume of fluid of high alkalinity, but the concentration of enzymes is not increased although their total quantity is considerably increased (p. 492).

Vagus stimulation results in a pancreatic secretion of small volume with an extremely high enzyme content. The vagus effect may be produced by administration of insulin (hypoglycemia), mecholyl chloride, pilocarpine or prostigmine.

EXAMINATION OF PANCREATIC JUICE— SECRETIN TEST^{1,2,5,7,15}

Quantitative analysis of the pancreatic juice should afford the most rational and accurate method of studying the external secretory function of the pancreas. Until recently this was unsatisfactory as a clinical procedure because of (a) inaccessibility of the secretion, (b) inability to obtain pancreatic juice

uncontaminated by variable amounts of bile and gastric juice and (c) lack of a satisfactory standard stimulus to pancreatic secretion. These difficulties have been overcome by the introduction of a purified preparation of secretin as a secretory stimulant and the use of a double-lumen gastroduodenal tube for the collection of pancreatic juice.¹

The long end of the tube is passed to the third portion of the duodenum, in which position the shorter end (ten inches shorter) is in the stomach. Continuous gentle suction is then applied, the negative pressure not exceeding 50 mm. Hg, and the gastric juice and duodenal contents are collected simultaneously. Usually after about twenty to twenty-five minutes the duodenal juice becomes clear and is no longer contaminated with gastric juice. The duodenal contents consist principally of pancreatic juice and usually some bile. When admixture with gastric juice has ceased, secretin is injected intravenously, in a dosage of 0.75 mg. per kilogram of body weight, and collection is continued for 1 hour.⁶ Subsequent collections may be separated into ten- and twenty-minute samples.

Immediately after injection of secretin, the volume of material aspirated from the duodenum increases strikingly, usually with a change in color. Prior to injection this is usually light yellow-brown unless the gallbladder is emptying. Promptly after stimulation of pancreatic secretion, either the color becomes paler or all trace of bile pigment disappears. In most instances the duodenal sphincter appears to remain closed and the liver bile accumulates in the gallbladder during the experimental period. The occurrence of a deeply bile-stained secretion throughout the test period suggests that the gallbladder is absent, diseased or nonfunctioning. Regurgitation of duodenal contents into the stomach occurs occasionally, especially in cases of achylia gastrica, when the pyloric mechanism is incompetent. This is evidenced by a sudden rise in the volume of a gastric fraction, by bile discoloration and by reduction in acidity of the fraction. By measuring the volume and the concentrations of bicarbonate and bilirubin, and comparing the latter with their concentrations in the corresponding duodenal fraction, the quantity of regurgitated material may be estimated with reasonable accuracy. The following quantitative determinations are made: (1) volume; (2) bicarbonate; (3) amylase; (4) trypsin; (5) lipase. Values are expressed as quantities excreted in the one-hour test period. Amylase is measured by the activity of digestion of starch, trypsin by digestion of protein (casein) and lipase by digestion of a fat substrate (e.g., olive oil). These are commonly expressed in terms of units of digestive activity.

Volume. In normal subjects, 135-250 cc. of pancreatic juice are obtained in the sixty-minute period, or 2.1-4.5 cc. per kilogram of body weight. In the majority of instances maximum secretion occurs during the first ten to twenty minutes, with a subsequent rapid fall, approximating the control rate of secretion in sixty to one hundred twenty minutes. The maximum rate of secretion varies from about 2 to 8 cc. per minute.

Bicarbonate. The normal total bicarbonate output in the one-hour period is 90-130 m. Eq., the maximum concentration occurring usually in twenty to forty minutes and falling more slowly than does the volume.

Amylase (Dlastase). About 300-1200 units of amylase are excreted in the one-hour period (5.5-11 units per kilogram of body weight). The curve of excretion reaches a peak during the first twenty minutes, falls sharply after twenty minutes and then gradually during the next one to two hours. The concentration of the enzyme is lower than in the resting juice except during the first ten minutes, during which the pancreas is probably emptying its store of preformed amylase. Subsequently, the stimulated gland forms a rather constant amount of enzyme, independent of the volume of secretion.

Trypsin. About 20-40 units are excreted normally in one hour (0.35-0.7 units per kilogram of body weight). What has been said of amylase applies to trypsin.

Lipase. The normal one-hour excretion is 7000-14,000 units (135-225 units per kilogram of body weight).

ABNORMAL FINDINGS WITH THE SECRETIN TEST

The general statement may be made that in case of obstruction of the pancreatic duct or extensive destruction of pancreatic tissue (*e.g.*, acute necrosis, carcinoma or fibrosis) there is usually a decrease in values for volume, bicarbonate and enzymes, under the conditions of the test, the decrease being proportional to the degree of duct obstruction or extent of tissue destruction. However, whereas this may be true of advanced lesions, such findings are not commonly observed in cases of mild pancreatic dysfunction. The earliest functional abnormality appears to be reflected usually in decreased excretion of amylase and lipase and later by a decrease in trypsin, the volume and particularly the bicarbonate being less easily disturbed. Nondissociated disturbance of function, as evidenced by a decrease in all factors, may perhaps be interpreted as indicating obstruction of the pancreatic duct or a significant diminution in the mass of functioning pancreatic tissue. Dissociated disturbance, first consisting usually in a decrease in amylase or lipase, the other

factors being relatively normal, represents the milder form of functional disturbance, and is seen characteristically in mild forms of acute and chronic pancreatitis.

Abnormal findings have been obtained with this procedure in patients with acute and chronic pancreatitis, pancreatic cysts, hemochromatosis, carcinoma of the pancreas and pancreatic edema. Abnormal results, indicating clinically unsuspected complicating pancreatic disturbance, have been obtained also in patients with diabetes mellitus, cholelithiasis, cirrhosis of the liver, acute hepatic necrosis and late syphilis. The procedure is of value in distinguishing steatorrhea of pancreatic origin from the idiopathic form (sprue, celiac disease), in which the secretin test yields normal results.

The response to insulin and mecholyl chloride has been utilized in the same manner as the secretin test. These agents normally produce a marked increase in the output (concentration) of pancreatic enzymes, the bicarbonate being unaffected. They have also been given in conjunction with secretin, the resulting output of enzymes being much greater than with secretin alone. However, normal values with the combined procedures have not been sufficiently well defined as yet and the findings in abnormal states appear to parallel those obtained with secretin alone.

EXAMINATION OF THE FECES

The determination of the presence or absence of pancreatic enzymes in the feces is of no practical significance. Perhaps the most valuable methods for the study of pancreatic function in the past have had for their basis the examination of the feces for evidence of inefficient fat and protein digestion. Fat is normally present in the feces in three forms, soap fat (combined fatty acid), free fatty acids and neutral fat, the relative proportion of each being dependent somewhat upon the efficiency of fat digestion and absorption. The normal values for each of these forms have been given elsewhere (p. 135).

Since pancreatic lipase splits neutral fat into fatty acids, the point of particular importance from the standpoint of the diagnosis of pancreatic functional efficiency should be the amount of unsplit fat present in the feces and the proportion this constitutes of the total fecal fat. From a consideration of the results obtained in a large series of cases Fowweather has reached the following conclusions:

(1) Any specimen in which the total fat amounts to more than 25 per cent of the total dry matter is probably abnormal.

The presence of excessive amounts of fat in the feces is termed "steatorrhea."

(2) Any specimen in which the neutral fat exceeds 11 per cent of the total dry matter or 55 per cent of the total fat should be suspected of showing evidence of deficient fat splitting.

(3) Any specimen in which the total split fat (*i.e.*, sum of soap fat and free fatty acids) exceeds 16 per cent of the total dry matter or 75 per cent of the total fat should be suspected of showing evidence of deficient fat absorption.

The following is an example of the results obtained by the analysis of fecal fat in chronic pancreatitis:

	Per cent of total dry matter
Total fat.....	42.1 (normal 17.5)
Soap fat (combined fatty acids) . . .	11.0 (normal 4.6)
Free fatty acids	5.8 (normal 5.6)
Neutral fat.. . . .	24.4 (normal 7.3)

The characteristic finding in this connection in pancreatogenous steatorrhea is a predominating increase in neutral fat, indicating deficient fat-splitting in the intestine. Characteristically, in severe cases, the feces are pale, bulky and foul-smelling, and, when allowed to stand, droplets or solid masses of neutral fat may be visible. This does not occur in celiac disease, sprue or idiopathic steatorrhea, in which the major increase in fecal fat occurs typically in the fatty acid fraction because of inadequate absorption. However, the findings are so similar in many cases of pancreatitis, celiac disease, sprue and idiopathic steatorrhea as to make the differentiation between pancreatitis and the other conditions impossible on this basis. According to Thaysen,²³ only an excessive loss of neutral fat (more than 35-40 per cent) points to pancreatic insufficiency. The secretin test (p. 493) is of much greater value in the differential diagnosis of these conditions.

In the presence of jaundice associated with pancreatic disease the values for all of the fatty constituents of the feces are considerably increased, the total fat in some cases constituting as much as 75 per cent of the total dry matter. In such cases there is evidence of disturbance of both fat digestion and fat absorption. Disturbances of intestinal motility, particularly those characterized by the rapid transit of food through the small intestine, are associated with alterations in the fat content of the stool. However, in such cases the fault lies primarily and principally in deficient fat absorption, and the proportion of neutral fat is usually within normal limits.

As stated elsewhere, doubt has been cast upon this interpre-

tation of variations in the relative proportions of the fatty constituents of feces due to the demonstration that fats are excreted in relatively large amounts by the intestinal mucosa.¹⁹ It appears probable, for example, that the enormous increase in fecal fat in obstructive jaundice may be due largely to an increased excretion of fat rather than to diminished absorption, as was formerly believed to be the case. Moreover, as the excreted endogenous fat is in the form of neutral fat and fatty acids, it is obviously impossible to interpret alterations in the relative proportions of these substances in the feces in terms of altered digestion and absorption of ingested fat.

Protein in Feces. In the normal individual not more than 5-10 per cent of the nitrogen of the food is lost in the feces. The term "azotorrhea" is used to indicate the excretion of excessive quantities of nitrogenous compounds in the feces, a condition dependent upon either imperfect digestion of proteins or imperfect absorption of protein derivatives. In the absence of abnormalities of absorption or intestinal motility, azotorrhea may be suggestive of pancreatic functional inefficiency. The presence in the feces of 25 per cent or more of the nitrogen of the food is frequently observed in pancreatic disease, whereas values above 17 per cent are rarely observed in sprue or idiopathic steatorrhea. In patients with steatorrhea maintained on a diet low in fat, a daily excretion of more than 3 Gm. of nitrogen suggests pancreatic dysfunction.

The stools of normal individuals may contain a few meat fibers with rounded ends and no transverse striations. Pancreatic enzymatic activity appears to be essential for digestion of meat fibers to proceed to the point at which the transverse striations disappear. Creatorrhea is a term applied to the presence of excessive quantities of undigested meat fibers in the feces, the fibers having sharp angular ends and transverse striations. In the presence of normal intestinal motility, creatorrhea is suggestive of impaired pancreatic function. In order to obviate, so far as is possible, misinterpretations due to too rapid or too slow passage of material through the intestines, all purgatives must be withheld and carmine and charcoal marked meals administered, only feces passed within from eighteen to thirty hours after ingestion of the corresponding food being accepted for examination. Abnormal findings on microscopic examination (undigested meat fibers, starch granules and excessive fat), because of their qualitative nature, are not in themselves diagnostic, but should serve as an indication for more exact chemical studies of the feces and for other tests of pancreatic function.

The Schmidt cell nuclei test, based upon the belief that cell nuclei are digested only by trypsin, and the Sahli glutoid capsule test, based upon the principle that gelatin capsules hardened in formalin are not dissolved in the stomach but are acted upon by pancreatic juice (trypsin), have been proposed as methods for the estimation of pancreatic functional efficiency but have not proved to be of practical clinical value.

SERUM AMYLASE (DIASTASE)

Blood normally contains a starch-splitting enzyme (amylase, diastase), which is also present in urine, lymph, feces and milk. Although apparently identical with salivary and pancreatic amylase,²² its origin is not known, since it is maintained at an approximately normal level after extirpation of the pancreas and salivary glands. However, certain acute pathologic changes in these glands may result in temporary diffusion of amylase into the blood. This phenomenon has proved of value in the diagnosis of acute pancreatitis. The method advocated by Elman⁹ for the quantitative determination of amylase consists essentially in the determination of the amount of sugar formed by incubation of definite quantities of blood and starch suspension. The result is expressed in milligrams of sugar formed by the action of 100 cc. of blood. Normally, 70–200 mg. of sugar will be formed by the action of 100 cc. of blood, according to this procedure. The method of Somogyi,²¹ which is in common use, is also based upon the amyloclastic activity of the blood serum, but measures the time required for complete depolymerization of starch, using iodine as an indicator. The results are expressed in terms of units of diastatic activity which are comparable in magnitude to milligrams of glucose, as expressed above. The diastatic activity of normal serum or plasma by this method is 60–180 units per 100 cc., 80 per cent of normal subjects falling between 80 and 150 units. Values above 200 and below 60 units are distinctly abnormal.

The chief clinical value of serum amylase determinations lies in the diagnosis of acute pancreatitis, whether the lesion is of the edematous, hemorrhagic, suppurative or necrotic type. In this condition, the serum amylase practically invariably increases almost simultaneously with the onset of symptoms, usually rising above 500 units and occasionally to over 3000 units, the peak being reached usually in twelve to twenty-four hours, but occasionally as late as forty-eight hours. After reaching the peak, there is usually a precipitous but occasionally a gradual fall to a normal or subnormal level within two to six days after the onset. The absence of an increase in serum amylase

within the first six to twenty-four hours after the onset of acute symptoms almost certainly excludes the possibility of acute pancreatitis as the cause of the symptoms, except in rare instances of extremely rapid and extensive destruction of acinar cells. Because of the rapidity with which normal values are restored, negative findings after forty-eight to seventy-two hours do not exclude this possibility. With the possible exception of serum lipase determinations, this procedure constitutes the most valuable method available for the diagnosis of acute pancreatitis.^{9,12,13,14} The increase is probably due mainly to inflow of preformed amylase from the damaged pancreas directly into the circulation via the portal vein and to a smaller extent via the lymphatics and thoracic duct.¹⁶

An increase in serum amylase has been reported in cases of peptic ulcer perforating into the pancreas, the values being considerably lower than in acute pancreatitis.¹⁸ Moderate increase has been observed occasionally in certain diseases of the salivary glands, including mumps, suppurative parotitis and calculous obstruction of the salivary duct.^{8,12} Moderate elevation (200-1000 units) may occur also as a result of renal functional impairment, due to deficient excretion of amylase. Subnormal values have been reported in subjects with severe hepatocellular damage (p. 463).

Normal findings are the rule in chronic pancreatic lesions (carcinoma, atrophy, chronic pancreatitis). Recent experiments in animals suggest the possible clinical value of serum amylase (and lipase) determinations after injection of secretin and mecholyl chloride as a test for pancreatic insufficiency. Under normal conditions, injection of adequate amounts of these substances, in combination, is followed by an increase in serum amylase (and lipase). No increase was observed with the same dosage in dogs with pancreatic atrophy resulting from duct ligation.¹⁷

SERUM LIPASE

The degree of lipolytic activity of the serum may be determined by the amount of olive oil hydrolyzed by a given quantity of serum in a given time. According to the titration method advocated by Comfort,³ the values for lipase are expressed in terms of the amount of N/20 NaOH per 1 cc. of serum. The upper limit of normal has been found to be about 1.5 cc. of N/20 NaOH.

Increase in serum lipase is believed to be indicative of pancreatic disease, the enzyme entering the blood stream in the same manner as pancreatic amylase. The findings with regard to lipase differ in certain important respects from serum amylase

in pancreatic disease. As in the case of the latter, in acute pancreatitis the serum lipase increases promptly at the time of onset of symptoms, values as high as 10.2 cc. having been reported. The subsequent fall is much more gradual than in the case of amylase, elevated values persisting in some cases for ten to fourteen days or longer. Because of this fact, accurate diagnosis is often possible by means of lipase determinations after the serum amylase has fallen to an essentially normal level. This procedure is preferred by some for this reason. In contrast to the usual normal levels of serum amylase in chronic disease of the pancreas, increase in serum lipase has been reported in about 40 to 50 per cent of cases of carcinoma of the pancreas, about 60 per cent of cases of carcinoma of the ampulla of Vater, 10 to 15 per cent of cases of chronic biliary tract disease (common duct stone or stricture, carcinoma of the bile ducts, cholangitis), and occasionally in duodenal ulcer, hepatitis and cirrhosis of the liver and chronic pancreatitis or pancreatic duct obstruction. In conditions not primarily involving the pancreas, increase in serum lipase is believed to indicate secondary pancreatic involvement. In cases of long-standing chronic pancreatic disease, with normal serum lipolytic activity, failure to obtain a rise by stimulation with secretin and mecholyl may prove to be of diagnostic value.¹⁷

AMYLASE IN URINE^{4,20}

Wohlgemuth found that urine normally contains 8 to 32 units of diastase (amylase) per cubic centimeter. Its immediate source is the blood amylase and, in the absence of significant impairment of renal function, variations in amylase in the urine tend to parallel those in the blood. Usually, in acute pancreatitis, the urine values rise shortly after those in the blood, the increase persisting for one to three days, and then fall sharply to normal. Values as high as 8000 units have been reported in acute pancreatitis, 300 units or more being of diagnostic significance.¹⁰ Elevated values have been obtained in chronic pancreatitis (during acute exacerbations), stone in the common bile duct, duodenal ulcer perforating into the pancreas, obstruction of the pancreatic duct and occasionally in carcinoma of the pancreas.

The following facts should be kept in mind in connection with amylase findings in blood and urine:⁴

(a) As in the case of serum amylase, much of the difference of opinion regarding the value of urinary diastase determinations has been due to failure to appreciate the transitory nature of the increase in acute pancreatitis.

(b) Excluding disease of the salivary glands and, in the

case of serum amylase, renal insufficiency, an increase is indicative of pancreatic disease, but this may be primary or secondary to or associated with such conditions as stone in the common duct, tumor of the common duct, stomach or duodenum, hepatic disease or duodenal ulcer.

(c) These procedures give no information regarding the nature or severity of the pancreatic lesion.

(d) Normal values do not invariably rule out pancreatitis, since they may be obtained when rapid extensive destruction of acinar cells has occurred.

CAMMIDGE REACTION

Cambridge found that in a large proportion of patients with acute and subacute pancreatitis, and in about 40 per cent of patients with chronic pancreatitis, the urine contained some substance which upon hydrolysis yields a pentose capable of forming an osazone. This unknown substance is believed to be in the nature of a glyconucleoprotein. Because of the high pentose content of pancreatic tissue and because of its more ready combination and liberation than pentose in other tissues, the Cambridge reaction is accordingly more consistently obtained in active degenerative lesions of the pancreas than in similar lesions involving other organs. However, careful studies reported by a number of investigators have not substantiated the clinical value of this test as claimed by Cambridge. A strongly positive reaction may aid in establishing a diagnosis of acute pancreatitis in the presence of clinical signs or symptoms of that disorder.

In cases of suspected pancreatic disease, in addition to measures designed to investigate the external secretory function of the pancreas, tests should be applied which yield information regarding the state of carbohydrate metabolism, which may be affected in a small proportion of cases through impairment of pancreatic islet function. Hyperglycemia and diminished glucose tolerance occur in about 50 per cent of cases of acute pancreatitis and glycosuria in about 10–15 per cent, more commonly in the hemorrhagic and necrotic forms of the disease than in pancreatic edema. Significant disturbance of carbohydrate metabolism is unusual in chronic pancreatic diseases (malignancy, inflammation, fibrosis) except in advanced cases with involvement of virtually the entire organ, as in hemochromatosis.

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Chapter XXII

Cerebrospinal Fluid^{2,3,4}

PERMANGANATE INDEX

THE permanganate or organic index is a figure expressive of the total amount of organic matter in the cerebrospinal fluid as measured by the reduction of a solution of potassium permanganate of known strength.

The normal index for fluid withdrawn by lumbar puncture is 1.3 to 1.8, ventricular and cisternal fluids having indexes which are slightly lower, 1.1 to 1.5. A most marked increase occurs in organic disease of the meninges. High values are found in all disorders associated with increase in the cell and protein content of the fluid. Contamination of the fluid with blood invalidates the results of this test. In general the index parallels the cell and protein content of the fluid. The determination of the permanganate index adds little to the information gained from other, more specific procedures.

PROTEIN

The protein content of cerebrospinal fluid is lower than that of any other normal body fluid with the exception of the aqueous humor of the eye, which closely resembles the cerebrospinal fluid in chemical composition. The amount of protein in normal fluid obtained by lumbar puncture is 15-45 mg. per 100 cc., cisternal fluid containing 10-25 mg. per 100 cc. The appearance of increased amounts of protein in the fluid is dependent upon the same factors which govern the presence of protein in all body fluids, normal and pathologic. Chief among these is the permeability of the barrier interposed between the plasma and the subarachnoid space, which, under normal circumstances, may be considered to be the choroid plexuses. Normally these vessels, as well as those of the meninges, are practically impermeable to proteins; in inflammatory conditions of the brain, cord and meninges the capillary walls become more permeable, and, depending upon the degree of inflammation, increasing amounts of albumin, pseudoglobulin, euglobulin and fibrinogen pass into the subarachnoid space, the last two appearing only in severe inflammatory processes.

Increase in the protein content may be found in conditions, other than inflammations, in which capillary and cell permeability are increased. These include: toxic states such as uremia, pneumonia and typhoid fever; convulsive states, as epilepsy and spasmophilia; conditions causing generalized or localized elevation of subarachnoid tension, as brain and spinal cord tumor.

Since in most pathologic fluids containing an increased quantity of protein globulin is present in abnormally high concentration, globulin tests are commonly used as a means of roughly estimating the protein content of cerebrospinal fluid. The most popular of these tests are the Noguchi, Nonne-Apelt, Ross-Jones and Pandy reactions; the results are reported as normal, 1 plus, 2 plus, 3 plus and 4 plus, depending upon the amount of globulin precipitated. More exact quantitative determinations may be made, the results being recorded in milligrams per 100 cc. of fluid.

Meningitis. Inflammatory exudates in any situation contain comparatively large amounts of protein. In the various forms of suppurative meningitis the total protein content of the cerebrospinal fluid is high (125-3000 mg. per 100 cc.), consisting of globulin, albumin and, at times, small amounts of fibrinogen. High values are also the rule in tuberculous meningitis (200-2000 mg.) and in acute luetic meningitis (180-540 mg.). In true meningitis the increase in protein usually parallels the increase in cellular content. It must be realized that in some cases of early meningitis the fluid protein may be normal. According to some observers, the determination of qualitative differences in the cerebrospinal fluid protein may be of value in distinguishing between tuberculous and other forms of meningitis. The Levinson test consists essentially in the comparison of the amount of precipitation produced in the cerebrospinal fluid by mercuric chloride and sulfosalicylic acid. A precipitate volume ratio of 2 to 1 or higher (bichloride precipitate: sulfosalicylic acid precipitate) is regarded as highly suggestive of tuberculous meningitis under the conditions of the test. The general experience is that this test is of only corroborative value and that it has little if any specific diagnostic significance.

Serous Meningitis. This term is applied to a condition of meningeal irritation of a noninfective nature which may be due to toxic states such as pneumonia, influenza, typhoid fever, and the like, or uremia, or to inflammation of structures adjacent to the cranial cavity, i.e., otitis media and mastoiditis.

The cerebrospinal fluid in serous meningitis or meningismus is, in most cases, entirely negative except for some increase in

amount and pressure. However, in isolated cases, abnormally high concentrations of protein may be found (18-70 mg.), other chemical and cytologic findings being normal. This protein increase may be comparatively slight and not detectable by the routine procedures for globulin determination.

Convulsive States. During and shortly after epileptic seizures there may be an increase in cerebrospinal fluid protein. Several investigators have reported increased albumin in the fluid in this condition. In the interval between convulsions the fluid is essentially normal. The same is true of the convulsions of spasmophilia of children. The protein concentration rises also during uremic convulsions.

TABLE 16
PROTEIN IN CEREBROSPINAL FLUID

Condition	Spinal fluid*	Cisternal fluid*
Normal.....	15- 45	
Tabes.....	40- 200	
Paresis.....	30- 190	
Cerebrospinal lues.....	20- 160	
Pneumococcic meningitis.....	150-2000	
Meningococcic meningitis.....	180-3000	
Streptococcic meningitis.....	160-2000	
Tuberculous meningitis.....	200-2000	
Brain tumor.....	30- 500	
Brain abscess.....	30- 500	
Froin syndrome.....	80-3000	25-180
Epidemic encephalitis.....	50- 300	
Cerebral hemorrhage.....	20- 220	
Cerebral thrombosis.....	20- 140	
Epilepsy.....	15- 80	
Serous meningitis.....	20- 70	
Myxedema.....	60- 200	

* Expressed in milligrams per 100 cc.

Organic Disease of Brain and Cord. Protein is increased in many organic diseases of the brain and cord, with or without associated pathologic change in the meninges. In the luetic affections, paresis, tabes and cerebrospinal syphilis, values of 20 to 200 mg. per 100 cc. are found, usually accompanied by an increase in cells. In epidemic encephalitis the protein ranges from 50 to 300 mg. per 100 cc. In cerebral hemorrhage, thrombosis and embolism the protein content may be normal or increased (20-220 mg.). In brain abscess without meningitis and in brain tumor with increased intracranial pressure the protein varies from 30 to 100 mg. per 100 cc. with slight or no cellular increase. The highest values for protein are found in

fluids presenting the so-called "Froin Syndrome" (xanthochromia, greatly increased protein, spontaneous coagulation and mononuclear pleocytosis). This syndrome is characteristic of spinal cord compression, due usually to tumor, associated with retention of spinal fluid in a cul-de-sac. Values of over 2000 mg. per 100 cc. have been reported in this condition. The protein content of the fluid above and below the point of obstruction may show marked differences in protein concentration. In one series of cases the protein content of the fluid removed by cisternal puncture was 24-180 mg. per 100 cc. while that obtained by lumbar puncture contained 80-3000 mg. per 100 cc. Such findings are of considerable diagnostic importance.

Myxedema. The protein content of cerebrospinal fluid is rather consistently increased in myxedema. Values as high as 100 mg. per 100 cc. are observed commonly and up to 200 mg. occasionally.

GLUCOSE

The concentration of glucose in the cerebrospinal fluid is dependent upon. (1) the blood sugar concentration; (2) the permeability of the protective barrier, represented by the lining of the choroid plexuses and possibly of the capillary terminations of the cerebrospinal vessels which are surrounded by prolongations of the subarachnoid space; (3) the rate of glycolysis within the fluid. It has been fairly well established that, in the absence of disease of the brain, cord or meninges, the concentration of sugar in the cerebrospinal fluid is approximately 60-70 per cent of that in the blood within a wide range of values extending from moderate hypoglycemia (40 mg.) to moderate hyperglycemia (400 mg.). The normal range has been found to be from 40 to 70 mg per 100 cc. in the fasting adult. The normal values for children up to ten years of age have been found to be 70 to 90 mg. per 100 cc. It has been shown that the fluid must be withdrawn under fasting conditions, and, in order to be properly interpreted, must be compared with the sugar content of the blood withdrawn at the same time. There is a delayed postprandial rise in spinal fluid sugar following that which occurs in the blood. Because of the lack of sufficient information regarding the blood and cerebrospinal sugar curve relationship following the ingestion of food, no significance should be attached to variations in the ratio of spinal fluid sugar to blood sugar except in the fasting state. The sugar content of ventricular and cisternal fluid is usually slightly higher than that of lumbar spinal fluid.

Practically all injuries to semipermeable membranes result in

an increase in their permeability. Hence, it is naturally inferred that, as with protein, any disease associated with injury to the cerebrospinal vascular system, particularly the choroid plexuses and cerebrospinal capillaries, may result in an increase in the sugar content of the spinal fluid, a condition termed hyperglycorachia. A decrease in spinal fluid sugar, hypoglycorachia, is almost invariably dependent upon an increased rate of glycolysis, this being probably the only agency by which the sugar concentration can be lowered independently of a diminution in the sugar content of the blood. Increased glycolysis occurs most strikingly in the suppurative meningitides, and to a lesser degree in tuberculous meningitis and in some cases of luetic meningitis. It is due to the glycolytic action of either the leukocytes or the organisms present in the fluid.

Hyperglycorachia. Epidemic Encephalitis. A frequent finding in the cerebrospinal fluid of patients with acute epidemic encephalitis is an increased glucose concentration. The figures range from 70 to 110 mg. per 100 cc. in adults. The presence of a subnormal spinal fluid sugar concentration militates against the diagnosis of acute epidemic encephalitis, a fact of importance in view of the varied symptomatology of this condition and the difficulty occasionally encountered in differentiating it from other disorders, particularly tuberculous meningitis.

Syphilis of the Central Nervous System. Increased sugar concentrations are found in some cases of syphilis, chiefly in that form affecting the cerebrospinal vessels particularly, the vascular form of cerebrospinal syphilis with little or no meningeal involvement. The figures range from 70 to 110 mg. per 100 cc. In cerebrospinal syphilis of the parenchymatous type, or paresis, with slight meningeal lesion, the sugar content is normal as it is in most cases of tabes. In acute luetic meningitis, low normal or slightly subnormal values are obtained.

Increased Intracranial Pressure. High sugar values (70-110 mg.) are usually found in conditions causing marked increase in intracranial tension. These include brain tumor, convulsive disorders and brain abscess not associated with meningitis. Increased sugar concentrations are present in some cases of serous meningitis, particularly in that type associated with uremia.

Functional Mental Disorders. Certain psychiatric disorders, particularly dementia praecox, may be accompanied by hyperglycorachia (70-95 mg.). This finding is not constant and is of no diagnostic value in these conditions.

Diabetes Mellitus. As stated above, high spinal fluid sugar values may be due to hyperglycemia. Recognition of this fact

is essential for the proper interpretation of spinal fluid sugar determinations.

Hypoglycorachia. Suppurative Meningitis. In all cases of acute meningitis associated with polymorphonuclear pleocytosis sugar is either entirely absent or present only in very small amounts (0-25 mg. per 100 cc.). Improvement in the condition of patients with meningococcic meningitis following the administration of antimeningococcic serum is accompanied by a rise in the spinal fluid sugar concentration.

Tuberculous Meningitis. Hypoglycorachia is an invariable feature of the fluid of tuberculous meningitis (18-36 mg. per 100 cc.). Sugar is rarely entirely absent as in the suppurative meningitides. This finding is of importance from the standpoint of differential diagnosis, particularly, as stated above, from acute epidemic encephalitis, which may in other respects simulate it very closely.

Luetic Meningitis. In a comparatively small number of cases of syphilis of the central nervous system, particularly those involving primarily the meninges and associated with a marked pleocytosis, the sugar content of the fluid may be slightly decreased (30-40 mg. per 100 cc.).

TABLE 17
GLUCOSE IN CEREBROSPINAL FLUID

Condition	Sugar*
Normal fasting adult	40- 70
Normal fasting child (to ten years)	70- 90
Functional mental disease	70- 95
Lues of central nervous system.	30-110
Epidemic encephalitis.	70-110
Suppurative meningitis	0- 25
Tuberculous meningitis	18- 36
Brain abscess	70-110
Brain tumor	70-110

* Expressed in milligrams per 100 cc.

NONPROTEIN NITROGENOUS CONSTITUENTS

Practically all of the known nonprotein nitrogenous constituents of the blood plasma are represented in the cerebrospinal fluid. The concentration of these substances in the fluid differs from that in the blood, however, depending upon their degree of diffusibility. The total nonprotein nitrogen varies from 12.5 to 30 mg. per 100 cc. Urea, being one of the most diffusible constituents of the blood, exists in all body fluids in

practically the same concentration. The urea content of cerebrospinal fluid, expressed as nitrogen, is 6 to 15 mg. per 100 cc. The creatinine varies between 0.45 and 1.5 mg., amino acids from 1 to 4 mg., and uric acid from 0.25 to 1.0 mg. per 100 cc., the last being slightly higher in children (0.3-1.5 mg.). The residual or undetermined nitrogen normally is approximately 50 per cent of that of the blood, ranging from 2 to 6 mg. per 100 cc.

The determination of these constituents of the spinal fluid is in most instances of little practical benefit, either diagnostically or prognostically. With the possible exception of uric acid they are altered in few conditions other than nephritis and uremia. Uric acid has been reported in increased concentration in all forms of meningitis. In most cases of nephritis and uremia the ratio between the total nonprotein nitrogen of the spinal fluid and blood is maintained, values as high as 375 mg. per 100 cc. having been reported. As these values increase, the urea nitrogen fraction constitutes an increasingly large proportion of the total. Thus, in one case, with a total nonprotein nitrogen of 110 mg. per 100 cc., the urea nitrogen was found to be 96 mg. per 100 cc. Creatinine, uric acid and amino acids usually increase in proportion to their concentration in the blood. In some cases of uremia, strangely enough in the asthenic type, without convulsions, the undetermined nitrogen fraction is found to be increased out of all proportion to its level in the blood. In a few instances it constituted 70 to 75 per cent of the total nonprotein nitrogen which ranged between 240 and 375 mg. per 100 cc.

As stated above, at the present time little or no practical importance can be attached to the determination of these constituents of the spinal fluid. However, the findings in nephritis and uremia are of considerable interest in view of the insight which they afford into the concentration of the various non-protein nitrogen fractions in the tissues and body fluids as contrasted with that in the blood.

CHLORIDE

The chloride content of the cerebrospinal fluid is from 720 to 750 mg. per 100 cc., expressed as sodium chloride. Values in infants exhibit slightly more variation, ranging from 650 to 750 mg. per 100 cc. This increased concentration in the spinal fluid as compared with that in the plasma (570-620 mg.) is explained in part on the basis of the Donnan equilibrium governing the concentrations of ions on either side of a semipermeable membrane (the capillary walls of the choroid plexuses) when the

fluid on one side (plasma) contains molecules which are not diffusible (protein) (see p. 230). The spinal fluid chlorides have been found to be increased in some cases of nephritis, but are of neither diagnostic nor prognostic importance in that condition.

In most forms of meningitis the chlorides are decreased, the diminution being most marked in tuberculous meningitis. In the suppurative meningitides the values range between 600 and 700 mg. per 100 cc. In tuberculous meningitis the figures are quite consistently below 600 mg., usually being from 450 to 580 mg. per 100 cc. In luetic infections of the central nervous system, in epidemic encephalitis, poliomyelitis and in practically all other diseases of the brain and cord the chloride content of the cerebrospinal fluid is but little altered. Therefore the determination of chlorides is particularly useful in the differential diagnosis of tuberculous meningitis from conditions with which it may be confused, particularly epidemic encephalitis. The decrease in cerebrospinal fluid chloride which occurs in inflammatory conditions of the meninges is probably due in a large measure to the increased protein content of the fluid in those conditions, the more equal distribution of protein between the blood plasma and cerebrospinal fluid resulting in a more equal distribution of chloride in those fluids (see p. 262). The decrease in plasma chloride concentration, which occurs at times in meningitis, also plays an important part in the production of this phenomenon.

Variations in the chloride content of the blood plasma are reflected in corresponding changes in the chloride content of the cerebrospinal fluid. Consequently, in such conditions as lobar pneumonia and upper intestinal or pyloric obstruction, which are commonly associated with hypochloremia, the concentration of chloride in the cerebrospinal fluid is correspondingly decreased. The plasma chloride concentration must therefore be considered in interpreting subnormal values for cerebrospinal fluid chloride, particularly in acute infectious diseases such as pneumonia, in which the development of symptoms of meningeal irritation may arouse suspicion of the presence of meningitis.

INORGANIC PHOSPHATE

The inorganic phosphate content of cerebrospinal fluid averages approximately 30-50 per cent of that of blood serum, ranging between 1 and 2 mg. per 100 cc. in adults and from 1.5 to 3.5 mg. in children. It is increased in all forms of meningitis, in nephritis and uremia with phosphate retention and has been found to be increased in conditions associated with degenerative processes in the brain and cord, such as tumor, tabes and paresis.

The determination of the cerebrospinal fluid phosphorus concentration has no practical value.

CHOLESTEROL

Most investigators have found that the normal cerebrospinal fluid contains no cholesterol or minute traces only. An increase occurs in many diseases of the central nervous system, including meningitis (trace-12 mg.), brain tumor and abscess (5-15 mg.), cerebral hemorrhage (5-20 mg.). In the majority of cases of syphilis of the central nervous system the cholesterol content of the fluid is not appreciably increased. In various mental disorders figures ranging from 0.2 to 0.7 mg. per 100 cc. have been reported.

LACTIC ACID

The cerebrospinal fluid normally contains no lactic acid. If allowed to stand at room temperature, lactic acid is formed in the process of glycolysis of the glucose present in the fluid. In all conditions in which the sugar content of the fluid is decreased lactic acid will be found to be correspondingly increased. It exists in highest concentration, therefore, in the suppurative meningitides, and, to a lesser extent, in tuberculous meningitis. It has also been found in patients with uremia.

HYDROGEN ION CONCENTRATION

The hydrogen ion concentration of cerebrospinal fluid examined immediately after withdrawal is almost the same as that of the blood, the pH varying normally from 7.4 to 7.6. Levinson has found that upon standing, exposed to air, the alkalinity increases, due to the loss of CO₂ from the fluid. The alkaline reserve, as measured by the CO₂ combining power, is also practically identical with that of blood plasma (55-75 volumes per cent).

The pH of the fluid in tuberculous meningitis is usually within normal limits (7.4-7.6), the alkalinity increasing upon standing, as in normal fluid. In the suppurative meningitides the fluid is slightly more acid (pH 7.2-7.5), the increase being due, in all probability, to the presence of lactic acid. Upon standing, the acidity shows much less tendency to decrease than in the case of normal fluids, actually increasing in some instances (Levinson). This is perhaps due either to an increased rate of glycolysis or fermentation, or to increased production of CO₂ by the cells present in the fluid, balancing the loss of CO₂ which occurs upon standing exposed to air.

SODIUM, POTASSIUM, CALCIUM AND MAGNESIUM

Determination of the sodium and potassium content of cerebrospinal fluid is, as far as is known, of no practical value. Figures for sodium range from 300 to 380 mg. and those for potassium from 16 to 22 mg. per 100 cc., being essentially the same as the values for the concentrations of these elements in the blood plasma under normal and abnormal conditions.

The calcium content of normal cerebrospinal fluid varies from 4.5 to 5.5 mg. per 100 cc. It is increased in practically all cases of meningitis and epidemic encephalitis. This is due to the fact that in the presence of protein in tissue fluids their calcium content is increased by an amount proportional to the quantity of protein present, the added calcium being nondiffusible in nature and the diffusible fraction being essentially unaltered. The terms "diffusible" and "nondiffusible," as here employed, refer to the ability to pass through a semipermeable artificial membrane or the capillary wall. The cerebrospinal fluid calcium is apparently all in ionized form and, under normal conditions, is quantitatively identical with the diffusible fraction of serum calcium. This correspondence between values for cerebrospinal fluid calcium and diffusible calcium of the blood serum as determined by artificial membrane methods is, however, lost under conditions of abnormal serum calcium concentration. For example, marked decrease or increase in serum calcium produced by parathyroidectomy or administration of parathyroid hormone is accompanied by little or no change in the cerebrospinal fluid calcium concentration. Instances of hypoparathyroidism have been reported in which the serum and spinal fluid calcium concentrations were identical (4.5 mg. per 100 cc.). Low values for spinal fluid calcium may be obtained in patients with renal failure, the decrease being roughly proportional to the increase in spinal fluid phosphate concentration. Some observers believe that there may be some relationship between the cerebrospinal fluid calcium concentration and the manifestations of muscular hyperirritability in uremia, such manifestations occurring frequently with spinal fluid calcium values below 4 mg. per 100 cc. (p. 394).¹

Reported values for magnesium concentration of the spinal fluid are slightly higher than those for normal blood serum, averaging about 3.3 mg per 100 cc.⁵ It would appear that the spinal fluid magnesium concentration is independent of variations in its concentration in the blood serum. Variations in this element appear to have little practical significance. Increased,

normal and decreased values for spinal fluid magnesium have been reported in various forms of meningitis.

XANTHOCHROMIA

Xanthochromia is a term applied to a condition in which the spinal fluid exhibits a clear yellow color. In most cases the pigment responsible for this discoloration responds to the indirect van den Bergh reaction, suggesting its derivation from hemoglobin and its intimate relationship with bilirubin. In some cases in which this reaction is not obtained the color may be due to some intermediary pigment complex or perhaps to lutein. The intensity of color may be expressed in terms of van den Bergh units or milligrams of bilirubin by the indirect van den Bergh reaction or, more simply, by the estimation of the icterus index.

Xanthochromia may be due to hemorrhage into the subarachnoid space from any cause. If blood is present in the subarachnoid space for more than a few hours the cerebrospinal fluid, following centrifugation, will exhibit a clear yellow color above the layer of packed red cells. This observation is at times of value in differentiating between pathologic hemorrhage into the subarachnoid space and the introduction of blood into the cerebrospinal fluid by injury to the subdural venous plexus at the time of puncture. The development of the yellow color is due to the occurrence of hemolysis with subsequent hydrolysis of hemoglobin into a pigment closely resembling, if not identical with, bilirubin. If the blood has been present for more than a week the yellow color becomes much more intense and then gradually fades, due probably to reabsorption of the pigment, finally disappearing entirely in from one to two weeks, depending upon the extent and duration of the hemorrhage.

In mild and moderately severe icterus, bilirubin, because of its relatively poor diffusibility, does not pass into the cerebrospinal fluid, but in advanced icterus, particularly of the obstructive type, varying amounts of bilirubin may be present in the fluid, imparting a yellow-brown color.

Xanthochromia may occur in compression of the cord by intramedullary tumors or by extradural compression as in the case of tuberculosis of the vertebra; it is particularly frequently observed in tumors in the region of the cauda equina. Under such circumstances a meningeal pocket is formed and venous stasis occurs with consequent capillary hemorrhage into the cerebrospinal fluid. A similar condition occurs in some cases of acute myelitis and in chronic meningitis with the formation of adhesions. In such cases, particularly in cord tumors, there is, in

addition, interruption of the cerebrospinal fluid circulation and transudation of serum in the area of venous and capillary stasis. The cerebrospinal fluid under such circumstances contains large quantities of protein. The term "Froin syndrome" is applied to xanthochromia in a fluid of high protein content which may coagulate spontaneously. This syndrome, because of its frequent occurrence in cases of tumor of the spinal cord, is of considerable diagnostic import.

Xanthochromia of varying degree occurs as a physiologic phenomenon in practically all premature infants during the first two months of life. About 60 per cent of such fluids respond to the indirect van den Bergh reaction, the icterus index being about 1 during the first twenty-four hours of life, increasing to reach a maximum of about 4 during the second week and then gradually decreasing to disappear in about eight weeks or earlier depending upon the degree of immaturity of the infant. In cases unassociated with hemorrhage into the subarachnoid space the xanthochromia of premature infants appears to be related to the increased bilirubin content of the blood serum existing during that period (icterus neonatorum). Since bilirubin appears in the cerebrospinal fluid of adults with icterus only when the latter is of severe grade or when there has occurred some damage to the choroid plexus, it appears that the permeability of the blood plasma-cerebrospinal fluid barrier in premature infants and in mature infants during the first few days of life is increased, due perhaps to the incomplete state of development of the cells constituting that barrier.

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Chapter XXIII

Biochemical Changes in Pregnancy and Lactation

METABOLIC changes which occur in the maternal organism during the period of pregnancy and labor are due in part to the metabolism of the fetus and in part to actual changes in certain phases of the intermediary metabolism of the mother incident to the pregnant state. Clinically significant findings with regard to the metabolism of sex hormones in normal and abnormal pregnancy are considered elsewhere (pp. 527, 534, 538, 543-551).

Basal Metabolic Rate. During the early period of pregnancy the basal metabolic rate is maintained within normal limits. During the later months, however, the basal metabolism rises and at term may reach an average value of +30. Some observers believe that this increase can be entirely accounted for by the metabolism of the fetus but others maintain that it is due in part to factors resident in the maternal organism, perhaps in the nature of increased thyroid and pituitary activity. The observation of an increased blood iodine concentration and increased excretion of iodine in the urine during pregnancy suggests that the increased basal metabolism during this period is dependent in part at least upon increased thyroid activity (p. 310).^{8,13} Increased values may persist throughout the period of lactation, being dependent in part perhaps upon the increased activity of the secreting mammary glands.

Blood and Plasma Volume. The blood volume increases steadily during pregnancy, the increase beginning in the first trimester and progressing until term, at which time the blood volume increment averages about 25 per cent.^{10,30} The plasma volume increases slightly more than the total blood volume, resulting in a slightly diminished concentration of the formed elements of the blood. Some believe that the mild anemia of normal pregnancy may be explained solely on this basis, but others feel that additional factors are operative, particularly iron deficiency and vitamin B₁ deficiency. The excess fluid is apparently lost rather rapidly following birth of the child, normal values for blood and plasma volume being restored at eight to ten weeks postpartum.

Protein Metabolism. During the last four months of pregnancy, as the protein requirements of the fetus increase, the mother is in a state of positive nitrogen balance, the urinary nitrogen being diminished in proportion to the protein intake. The quantity of protein stored appears to be greatly in excess of the needs of the fetus, and, indeed, a distinctly positive nitrogen balance may exist in the early months of pregnancy, at which time it cannot be accounted for on the basis of fetal requirements alone.^{19,20} Following parturition, the nitrogen balance becomes negative, due partly to loss of protein incident to involution of the uterus and partly to the elimination of relatively large quantities of protein in the milk.

A mild degree of albuminuria occurs in 30 to 50 per cent of women during normal pregnancy and labor, disappearing immediately after parturition in the absence of complicating factors. This is attributed by some to purely mechanical factors, such as lordosis and the weight of the gravid uterus, causing pressure on the inferior vena cava and congestion of the kidneys. However, no definite statement can be made in this connection.

The concentration of plasma protein is rather consistently diminished during pregnancy, the diminution occurring chiefly or entirely in the albumin fraction, the plasma fibrinogen and globulin increasing progressively during the later months of pregnancy and in labor, returning gradually to normal during the puerperium. The average decrease in albumin and total protein concentration in the late months of pregnancy is approximately 1 Gm. per 100 cc. in uncomplicated cases, the amount of decrease being somewhat greater in women maintained upon a low protein diet.³⁶ This decrease in plasma protein concentration is generally attributed largely to the state of hydremia (relatively high plasma volume) which exists during this period. However, there is evidence that the store of reserve protein may be diminished and that the ability of the organism to regenerate serum protein may be markedly impaired during pregnancy and lactation. It is well recognized that the fetus draws upon the mother for its dietary factors, including its protein precursors, probably amino acids. As stated by Melnick, the synthesis of body protein in the fetus during pregnancy and of milk protein during lactation may be regarded as an internal plasmapheresis, leading to depletion of the maternal serum protein. He believes that these parasitic effects on the maternal organism are of primary importance, over and above the factor of hydremia, in causing the characteristic decrease in serum protein concentration during pregnancy. In the absence of complicating factors, and in the absence of marked dietary restriction of protein, the

degree of reduction of the colloid osmotic pressure of the plasma in pregnancy is usually not sufficient for the production of edema. However, the development of edema is favored, as a result of this change, in the presence of relatively slight abnormalities in other factors which contribute to the production of this phenomenon (p. 257).

Normal pregnancy is usually characterized by a decreased concentration of total NPN in the blood, a relatively greater decrease in the blood urea N and a subnormal ratio of urea N to total NPN.^{1,2,15} The lowest levels of NPN and urea N are usually attained during the sixth month of pregnancy, at which time the NPN ranges from about 19 to 27 mg. per 100 cc. and the urea N from about 5 to 10 mg. per 100 cc. Since the determinable nonprotein nitrogenous constituents of the blood, with the possible exception of uric acid, remain within approximately normal limits, the relatively marked drop in urea N as compared to the total NPN is due to an increase in the "rest nitrogen" or "undetermined" nitrogen. This fraction increases from the normal range of 5 to 18 mg. per 100 cc. to levels of 15 to 22 mg. per 100 cc. during the last three months of pregnancy. The NPN and urea N increase slightly after the sixth month. Although the majority of observers report little or no alteration in the blood uric acid concentration in normal pregnancy, we have observed values as high as 5.5 mg. per 100 cc. (whole blood) in apparently normal pregnant women.³

Voge and subsequent investigators found that histidine, an amino acid, is absent from normal urine and is present in the urine in the early stages of pregnancy.^{14,24,38} It appears at about the fifth week of gestation, continues throughout the remainder of pregnancy and disappears from the urine within a few days after delivery. There is some evidence to support the view that the activity of liver histidinase is inhibited during pregnancy, failure of this enzyme to catalyze the catabolism of histidine accounting for the presence of this amino acid in the urine. The amount of histidine is increased by a high protein diet.

Renal Function. By means of the water function test, it has been found that the capacity of the kidneys to handle water diminishes progressively during the last three months of normal pregnancy.²³ However, this may be dependent primarily upon the decrease in plasma protein rather than upon a disturbance in kidney function. Normal urea clearance values have been reported by most investigators,^{12,22} but subnormal findings may be obtained in the late months of pregnancy in women maintained on a diet deficient in protein (p. 375).⁴

Blood Lipids. The plasma cholesterol usually rises during the course of normal pregnancy. A maximum concentration is usually reached at about the thirtieth week, the average increase in free cholesterol at that time being about 25 per cent and that in ester cholesterol about 9 per cent. According to some, the free cholesterol diminishes subsequently and the ester fraction increases until an approximately normal ratio is reached just before parturition, at which time there is an average increase of about 25 per cent in the total plasma cholesterol concentration. The normal level is restored about eight weeks postpartum. There is a simultaneous increase in the concentration of neutral fat and fatty acids, which persists during pregnancy and may be more marked during lactation (p. 144). There is also an increase in plasma phospholipids, amounting at term to about 25 per cent of the normal nonpregnant values, with an additional increase occasionally during lactation (p. 145). No satisfactory explanation can be advanced at the present time regarding the mechanism of production of the lipemia of normal pregnancy. The available data suggest that it probably belongs in a similar group of lipemias represented by diabetes mellitus and experimental anemias.

Carbohydrate Metabolism. Glycosuria (see p. 63) occurs in from 10 to 15 per cent of all normal pregnant women, particularly in the later months, and more frequently in primigravida than in multigravida. The fasting blood sugar remains within normal limits during this period. The nonhyperglycemic glycosuria of pregnancy is ascribed by some to a diminution in the "renal threshold level" for glucose, a condition similar to renal glycosuria. Others believe it to be due to an actual decrease in glucose tolerance resulting perhaps from a state of physiologic hyperpituitarism or hyperthyroidism which may be present at this time. Lactosuria seldom occurs during normal pregnancy but is present in a considerable proportion of women during the period of lactation.

Diabetes mellitus may be precipitated and, if previously present, is frequently aggravated by pregnancy.⁹ A significant decrease in the glucose tolerance of diabetic pregnant women often occurs at about the sixth month of pregnancy.³⁴ In some cases there has been an actual improvement in the sugar metabolism of diabetics during gestation. An increase in glucose tolerance in such cases has been attributed by some to the transference of insulin from the fetus to the mother, the fetal pancreas partially compensating for the diminished production of insulin by the diabetic mother. However, Skipper believes that the reduction in glycosuria and insulin requirement in late

pregnancy and in lactation in some cases may be best accounted for by the passage of dextrose and dextrose-forming substances into the fetus rather than to the passage of fetal insulin into the maternal circulation.

Acid-base Balance. As pregnancy progresses there is a distinct tendency toward ketosis, which may be produced on carbohydrate-poor diets much more readily than in normal individuals. Under such circumstances ketonuria may occur. Beginning about the second month of pregnancy there is a gradual progressive decrease in total serum base, plasma CO_2 combining power, alveolar CO_2 tension and plasma bicarbonate.^{25,29} The plasma pH is usually within normal limits but some believe that it tends to be increased. There has been considerable controversy regarding the interpretation of these observations, centering about the point as to whether the fall in plasma bicarbonate is brought about by a primary CO_2 deficit or a primary alkali deficit (p. 281). Some believe that the fall in bicarbonate results from hyperventilation; *i.e.*, hyperventilation lowers the plasma CO_2 , the base falling secondarily as a compensatory phenomenon.²⁹ This compensation is usually adequate to prevent an increase in plasma pH , but in some cases a slight increase in pH may occur because of inadequate compensation. According to this view, the disturbance of acid-base balance in normal pregnancy is in the direction of an alkalosis, usually but not invariably compensated. Other observers deny the occurrence of increased plasma pH in normal pregnancy and believe that the decrease in plasma bicarbonate is due to a primary base deficit, constituting a state of acidosis which is usually well compensated so that the plasma pH remains within normal limits.²⁵ In our opinion, the bulk of evidence supports the latter view, although no definite statement can be made in this connection at the present time because of insufficient data.

Mineral Metabolism. It has been shown that during pregnancy calcium and phosphorus are retained by the maternal organism.^{5,7} The amount of retained calcium is more than can be accounted for by fetal utilization and perhaps represents the establishment of a reserve supply which may be called upon during subsequent emergencies. This storage of calcium is particularly marked in the later months of pregnancy and is associated with a tendency toward a diminution in the concentration of serum calcium. During the course of normal pregnancy and early labor there is a gradual diminution in the level of total serum calcium, which decreases from an average value of 10.6 mg. in the early months to 9.6 mg. per 100 cc. dur-

ing the first stage of labor. Some observers have reported values as low as 8 mg. per 100 cc. in normal women at term. The mechanism underlying these changes is not clearly understood. They are apparently not dependent entirely upon primary changes in serum phosphate or protein and may be due to physiologic changes in the functional activity of the parathyroid glands. This hypothesis is contradicted by the observations of Hoffmann and Hamilton, which suggests that the content of parathyroid hormone in the blood is higher than normal during the latter half of pregnancy. This increased secretion of parathyroid hormone is regarded as compensatory in nature. An increase in serum phosphatase activity has been observed during the last two months of pregnancy.²⁷ Although there appears to be a tendency for the serum inorganic phosphorus to decrease slightly during the late months of pregnancy, no significant deviation from the normal occurs in this factor. The interesting observation has been made that the concentrations of calcium and phosphorus in the cord blood (fetal circulation) are from 1 to 2 mg. per 100 cc. higher than in the maternal circulation and that the concentrations of these elements in the fetal circulation are dependent upon their concentrations in the maternal blood.²⁸ A slight increase in serum magnesium concentration has been observed in normal pregnancy, the significance of which is not known.

The transition from pregnancy to the period of lactation is characterized by a distinct alteration in mineral metabolism.²¹ This consists of a sudden change from the state of positive calcium and phosphorus balance to one of negative calcium and phosphorus balance, which persists during the period of lactation. The loss of calcium and phosphate is due not only to the large quantities of these elements present in the milk but also to excessive excretion, particularly in the feces, the quantity of calcium in the feces at times exceeding the total calcium intake.

Hepatic Function. Some degree of impairment of hepatic function, judged by normal standards, may be present in a relatively large percentage of women during pregnancy, particularly during the last few months.^{6,35,37} This can be demonstrated most frequently by means of the bilirubin excretion test (p. 449). The serum bilirubin concentration is rarely if ever increased above the upper limit of normal in normal pregnancy. A variable degree of impairment of the ability to excrete brom-sulfalein (p. 457) has been found occasionally in the late months. Studies of the chemical composition of gallbladder bile at term suggest that in pregnancy there is an alteration both in the composition of the liver bile and in the absorptive function

of the gallbladder.³³ There is apparently an increase in the cholesterol concentration of the gallbladder bile and a decrease in its bile salt concentration. These changes theoretically predispose to the formation of biliary calculi. The chloride concentration is higher and the calcium concentration lower than in normal gallbladder bile in the nonpregnant state.

TOXEMIAS OF PREGNANCY

Protein Metabolism. Albuminuria may occur in the pernicious vomiting of pregnancy and preeclamptic and eclamptic toxemias. It is usually most marked in eclampsia, but may, in rare instances, be absent in that condition. Some degree of edema, latent or frank, is almost invariably present in true toxemia of pregnancy. This has been shown to be associated with a decrease in the plasma protein concentration. This decrease occurs practically entirely in the albumin fraction and is more marked in eclampsia than in the nonconvulsive forms of pregnancy toxemias. As in the case of normal pregnancy, this decrease in plasma protein concentration is aggravated by deficient protein intake. Although the degree of hypoproteinemia may not in itself be sufficient to produce edema, it favors the development of this phenomenon in the presence of other contributing factors, such as an increase in capillary pressure (p. 257).³⁶

In mild and moderately severe cases of pernicious vomiting of pregnancy, the nonprotein nitrogenous constituents of the blood remain within normal limits. In severe cases, however, they may be increased. This increase may be associated with a state of hypochloremia which is at times dependent upon excessive and prolonged vomiting (see pp. 101, 235). The blood amino acids may be simultaneously increased. In preeclampsia and eclampsia the total NPN and urea N are usually within normal limits. Most observers report an increase in blood uric acid. This is frequently the only demonstrable abnormality in blood nonprotein nitrogenous constituents. In occasional cases with urinary suppression the nonprotein nitrogenous constituents of the blood may be considerably increased. Similar increases are also observed in (a) nephritis complicating pregnancy, which may be confused with eclampsia, (b) renal cortical necrosis, which at times occurs during or immediately following pregnancy, and (c) occasionally in severe hepatic necrosis with an associated "hepatorenal syndrome" (p. 101).

There may also be an alteration in the urinary nitrogen partition consisting of a decrease in the proportion of urea nitrogen (70-80 per cent total NPN), a relatively high ammonia content (5-10 per cent) and a high amino acid nitrogen content.

These changes are due in part perhaps to impairment of hepatic function and in part to the existing state of acidosis.

Renal Function. There is a distinct tendency at the present time to regard the hypertensive toxemias of pregnancy as manifestations of a generalized vascular reaction eventuating in chronic vascular disease in about 25 per cent of cases. The special predilection of pregnant women for the development of these manifestations may be dependent, in part at least, upon certain physiologic changes which occur during this period. Among these are physiologic hydronephrosis, which predisposes to pyelonephritis and prevents proper drainage and elimination of infection, and the physiologic hypoproteinemia, which enhances the tendency to edema. The blood urea clearance is usually within normal limits in eclampsia.^{12,22} It has been found, however, that reexamination of such cases about three months postpartum reveals a significant decrease in blood urea clearance in 25-50 per cent of cases, suggesting the presence of permanent renal or renal vascular damage.¹² Renal function tests are of particular value in this connection during pregnancy in distinguishing between true eclampsia and glomerulonephritis.

Carbohydrate Metabolism. Increased, decreased and normal blood sugar values have been reported in eclampsia. The majority of investigators adhere to the belief that the blood sugar in eclampsia, if altered at all, is slightly increased. There seems to be little doubt that the store of glycogen in the liver diminishes during pregnancy; the glycogenic function of this organ is undoubtedly further impaired in eclampsia, one important pathologic feature of which is an acute toxic degenerative process in the liver. It is probable that the variation in the reported blood sugar findings is due to the wide variation in the nutrition of toxic patients which cannot possibly be adequately controlled during the period of observation in active eclamptic states.

Lipid Metabolism. The concentration of blood lipids, including cholesterol, is essentially the same in eclampsia as in normal pregnancy (see p. 517).

Acid-base Balance. A marked state of ketosis may be present in the pernicious vomiting of pregnancy, as evidenced by ketonuria and a high concentration of acetone bodies in the blood, values of over 100 mg. per 100 cc. having been observed in some cases. This ketosis may be due to carbohydrate starvation which occurs as a result of the inability to retain food administered by mouth. In most cases the hydrogen ion concentration and the CO₂ combining power of the blood are not essentially changed. The plasma chloride concentration is

usually subnormal, probably as a result of prolonged vomiting. It may be that the tendency toward alkalosis caused by hyperventilation and the excessive loss of hydrochloric acid from the stomach is balanced by the tendency toward acidosis resulting from the loss of large quantities of fluid and base and the existing state of ketosis.

Eclampsia, if of severe grade, is almost invariably associated with a distinct diminution in the CO_2 combining power of the blood, which frequently falls below 30 volumes per cent and occasionally as low as 12 volumes per cent. Increased values for blood lactic acid have been reported. Some observers regard changes in acid-base balance in eclampsia as fundamentally identical with those in normal pregnancy, the decrease in serum base and bicarbonate and in the CO_2 combining power being assumed to be due primarily to hyperventilation, with secondary attempt at compensation for the loss of CO_2 . In view of the many complicating factors, such as vomiting and carbohydrate privation, it seems more logical to assume that the disturbance of electrolyte and acid-base equilibrium in eclampsia is dependent upon the symptoms rather than upon the fundamental character of the condition.

Mineral Metabolism. Subnormal, normal and increased serum calcium values have been reported in eclampsia. Some observers report an increase in serum phosphate, resulting in a decreased ratio of calcium to phosphorus.

Hepatic Function. The majority of reports suggest that some disturbance of liver function is a common manifestation of pregnancy toxemias. Hyperbilirubinemia, increased urobilinuria, some degree of bromsulfalein retention and impaired removal of injected bilirubin have been observed in this condition. However, critical comparison of observations in eclampsia and in normal pregnancy fail to reveal much significant difference between the findings under normal and abnormal conditions in the absence of other complicating factors.⁶ Evidence of impaired liver function is, of course, obtained usually in the presence of hepatic and biliary tract disease complicating pregnancy. Marked impairment of liver function, with jaundice, is frequently present in patients with acute or subacute hepatic necrosis, which some authorities believe to be one of the characteristic features of eclampsia.

Blood Guanidine. Some investigators have found the "guanidine" content of the blood of patients with preeclamptic and eclamptic toxemia to be increased. Similar observations have been made upon patients with acute hepatic disease such as acute yellow atrophy of the liver, arsphenamine hepatitis,

and carbon tetrachloride and chloroform poisoning, suggesting that the increase in blood "guanidine" in eclampsia is dependent upon the hepatic lesions in that condition. The significance of these observations is questionable in view of the lack of specificity of the methods employed for the determination of guanidine in the blood (p. 421).

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Chapter XXIV

Hormone Assay and Endocrine Function

A. E. Rakoff

THE functional state of some of the glands of internal secretion can be determined at the present time only by indirect methods, *i.e.*, by studying certain phases of metabolism which are influenced specifically by certain hormones. For example, the state of parathyroid function is most satisfactorily ascertained clinically by studies of calcium and phosphorus metabolism and serum phosphatase activity. Abnormal function of the pancreatic islet cells is reflected in abnormalities of carbohydrate metabolism, and thyroid function in the basal metabolic rate, cholesterol and carbohydrate metabolism and creatine metabolism. Water metabolism is affected by the antidiuretic hormone of the posterior lobe of the hypophysis and carbohydrate metabolism by pituitrin and epinephrine. Carbohydrate and protein metabolism are influenced by adrenal cortical hormones and the adrenotrophic hormone of the anterior hypophysis, and protein metabolism also by the male sex hormone and the growth hormone of the anterior pituitary. The adrenal cortex exerts a specific effect upon the metabolism of sodium, potassium, chloride and water. The manner in which these metabolic abnormalities may be used as an index of the state of function of various endocrine glands has been indicated elsewhere, in discussing the metabolism of each of the substances in question.

A more direct approach is possible in the case of certain hormones, quantitative determination of their concentration in the blood or urine affording a more or less exact index of the functional activity of the organs in which they originate or of those concerned with their intermediate metabolism. Thus, the concentration of organic iodine in the blood plasma or serum is a measure of the quantity of circulating thyroid hormone (p. 217). Assays can also be made of certain hormones of the anterior hypophysis, the ovary, testis and placenta and the adrenal cortex. The majority of these, at the present time, can be determined quantitatively only, or at least most satisfactorily, by bioassay, but adequate chemical methods are available in some cases. Because of the importance of this field of clinical

investigation, and because it is fundamentally biochemical in nature, the clinical significance of data obtained by such procedures will be discussed, even though the methods employed in most instances are biological rather than chemical.

ANTERIOR PITUITARY HORMONES^{35,36}

The anterior lobe of the pituitary gland secretes a number of so-called "trophic" hormones, *i.e.*, substances that stimulate and thus regulate the function and also maintain the structure of other glands of internal secretion. Among these are gonadotrophins (follicle-stimulating and luteinizing), thyrotrophin, adrenocorticotrophin, growth hormone, prolactin and, according to some, mammogenic and parathyrotrophic hormones.

THE GONADOTROPHIC HORMONES^{7,10,14}

The anterior lobe of the pituitary gland stimulates the ovary and testis by means of the following specific gonadotrophic hormones, which are believed to be secreted by the basophilic cells of this gland:

(1) Gametokinetic Gonadotrophin (Follicle-Stimulating Hormone, FSH; Prolan A; Thylakentrin). This, in the female, stimulates growth and maturation of ovarian follicles and estrogen production, and in the male, stimulates spermatogenesis.

(2) Interstitial Cell-Stimulating Hormone (ICSH) (Luteinizing Hormone, LH; Prolan B; Metakentrin). This, in the female, stimulates certain interstitial cells of the ovary to undergo luteinization and, in the male, stimulates the interstitial cells of Leydig to produce male sex hormone. There is some evidence that there may be two distinct interstitial cell-stimulating hormones, one which has to do with luteinization and another which stimulates the corpus luteum to secrete progesterone and estrogen. The latter gonadotrophic hormone is therefore sometimes referred to as the "luteotrophic" gonadotrophin.

The chorionic cells of the placenta in the human also produce a gonadotrophic hormone, which is termed "chorionic gonadotrophin." Superficially, it behaves much like the luteinizing hormone of the pituitary and is therefore frequently referred to as "anterior pituitary-like hormone" or "APL." It differs from the pituitary luteinizing hormone in that it will not produce luteinization in the hypophysectomized animal.

A fourth type of gonadotrophic hormone is produced by the placenta of the pregnant mare (equine gonadotrophin) and is present in the blood of that animal during pregnancy. It differs from human pregnancy gonadotrophin (chorionic gonadotrophin) in that it has both follicle-stimulating and luteinizing

properties, and also in that it is not excreted in the urine of the pregnant mare.

The gonadotrophic hormones are complex glycoproteins,^{7,14} which have not yet been isolated in pure form. They are soluble in water and may be precipitated by alcohol or tannic acid. They lose their potency on standing in solution and are readily destroyed by heat.

Physiologic Considerations

Although unquestionably gonadotrophins are secreted by the pituitary gland from the time of birth or earlier, detectable amounts do not appear normally in the urine until about ten to twelve years of age, being subsequently elaborated in increasing amount and producing gonadal stimulation and the phenomena of pubescence. In the female, after the menarche, the pituitary gonadotrophins are secreted in a cyclic fashion (Fig. 22). The follicle-stimulating type of gonadotrophin predominates during the first half of the menstrual cycle and the luteinizing during the last half, although there is evidence that both are present throughout the entire cycle. Shortly after, or possibly a few days before, the onset of the menstrual flow, the gonadotrophin concentration (blood and urine) slowly rises. As the midcycle is approached, there is generally an increase in gonadotrophin concentration in the blood and urine. A sharp peak is commonly reached just before ovulation; in some women no such sudden rise can be observed, while in others more than one peak may occur between the seventh and twenty-first days of the cycle (Fig. 22 and Table 18). Under the influence of the rising concentration of gonadotrophin, one or more primordial follicles are stimulated to grow and to undergo maturation. As a rule, only one of the follicles ripens completely in preparation for ovulation. Apparently a proper synergistic mixture of the follicle-stimulating and luteinizing hormones must be present in order to furnish the necessary stimulus for normal ovulation. The prevalent belief is that ovulation, in the human, occurs about fourteen days before the next menstrual flow, regardless of the length of the cycle. Following ovulation, the corpus hemorrhagicum is converted to a corpus luteum under the influence of the luteinizing gonadotrophin. During the next ten days the corpus luteum grows and becomes more active functionally but, as the gonadotrophic activity diminishes in the premenstrual phase, this structure degenerates and becomes a corpus albicans unless pregnancy has supervened. Thus, under the influence of the gonadotrophic hormones of the hypophysis, the ovary has passed through two endocrine phases: (1) the

growing follicle, which produces increasing amounts of estrogen and culminates in ovulation; (2) the corpus luteum, which produces both progesterone and estrogen, preparing the endometrium for pregnancy.

THE FEMALE SEX ENDOCRINE CYCLE

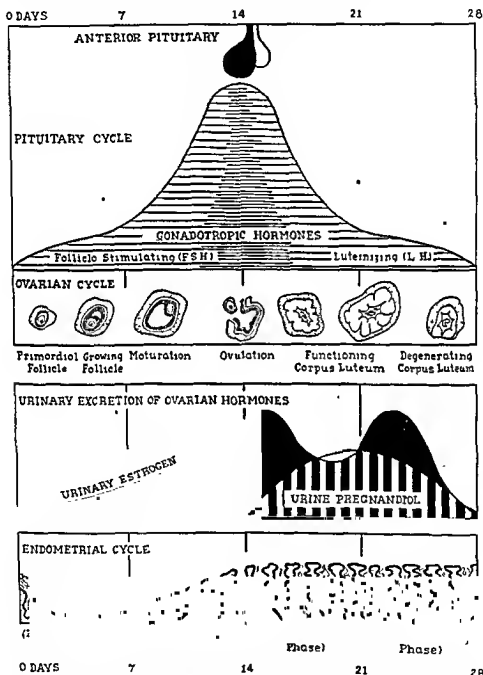


Fig. 22.—The female sex endocrine cycle. (Courtesy of Dr. A. E. Rakoff.)

Pregnancy. During pregnancy the gonadotrophin content of the blood and urine increases enormously because of the production of huge amounts of chorionic gonadotrophin by the placenta. It is believed that the pituitary gonadotrophins are

almost completely suppressed throughout the period of gestation. Chorionic gonadotrophin enters the maternal blood stream as soon as the chorionic villi have established contact with the maternal circulation, which usually occurs during the last week of the cycle. This increase in luteinizing factor causes the corpus luteum to persist and grow as the corpus luteum of pregnancy, which continues to function during the first trimester

TABLE 18
SEX HORMONE VALUES IN URINE AND SERUM

	Female, days of the menstrual cycle				Meno- pausal women	Male
	7	14	21	28		
GONADOTROPHIN ¹						
Urine ¹						
Range	Traces-12	8-40 ¹	Traces-8	0-6	32-300	4-24
Average	6	20	6	Traces	80	8
Serum ^{1a}	0-traces	Traces-3	0-traces	0	3-30	0
ESTROGEN ¹						
Urine ¹						
Range	65-160	160-660 ¹	160-660 ¹	30-110	10-65	25-100
Average	110	330	300	65	Under 65	50
Serum ^{1a}	Traces-6	3-9	3-9	0-3	0-traces	
PREGNANOIOL						
Urine ¹						
Range	0	2-6	3-10	0-4	0	0
Average	0	4	6	Traces	0	0

¹ In mouse uterine-weight units per 24 hours

^{1a} In mouse ovarian-weight units per 100 cc.

² In international units per 24 hours, assay made on castrate mice using vaginal

ann.

peak in one cycle.

³ Two peaks in estrogen excretion are generally observed, one at the time of ovulation and another four or five days premenstrual.

of pregnancy. Chorionic gonadotrophin rises rapidly (blood and urine) during the early weeks of gestation to reach a very high peak, usually between the sixth and twelfth weeks, falling off subsequently to more moderate levels at about the twentieth week, after which time the gonadotrophin concentration remains fairly constant for the remainder of the period of gestation (Figs. 23 and 24, Table 19). The early, rapid rise in gonadotrophic hormone has furnished the basis for a number of popular

tests for pregnancy, including the Friedman, Aschheim-Zondek, South African frog and six-hour rat tests.

The gonadotrophic hormone disappears rapidly from the blood and urine following delivery of the placenta, and normally is almost entirely absent within a week postpartum. It is believed that during the puerperium, lactogenic hormone (pituitary) blocks the liberation of pituitary gonadotrophins and thus results in a variable period of amenorrhea and low fertility during lactation.

Menopause. With the approach of the menopause, the ovary becomes progressively less responsive to gonadotrophic stimulation, with a consequent increasing functional demand upon the pituitary for gonadotrophic hormone, chiefly of the follicle-stimulating type. As estrogen production by the ovary continues to diminish, an increasing concentration of follicle-stimulating gonadotrophin appears in the blood and urine of the menopausal woman. The high gonadotrophin titer may persist for a variable number of years.

Gonadotrophins in the Male. Gonadotrophins are usually demonstrable in the urine of boys of about eleven to thirteen years of age, increasing gradually to reach a stable level by fourteen years of age. There is no cyclic rhythm in the urinary excretion of gonadotrophins, which are both follicle-stimulating and luteinizing in nature. A moderate increase in gonadotrophins occurs at times in men after the age of fifty years (male climacteric).

Determination of Gonadotrophic Hormones

When present in high concentration, as during pregnancy, gonadotrophins can be assayed by direct injection of blood or urine in suitable laboratory animals. In the normal, non-pregnant state, however, they are present in such small amounts that the urine or blood must be concentrated or extracted before the assay. The most commonly employed methods of gonadotrophin assay are based upon the effects of injection of urine, serum or partially purified extracts of these fluids into immature rodents. Several biologic end-points have been employed: (a) increase in weight of the uterus; (b) increase in weight of the ovaries; (c) production of corpora lutea; (d) vaginal estrus; (e) increase in weight of the seminal vesicles. The choice of method depends mainly upon the type and amount of gonadotrophin present. Results obtained by different laboratories must be interpreted on the basis of normal values obtained with the methods and technic employed.

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ESTROGENS						
Urine ³						
Range	65-160	150-660 ³	160-660 ³	30-110	10-65	25-100
Average	110	330	300	65	Under 65	50
Serum ^{2a}	Traces-6	3-9	3-9	0-3	0-traces	
PREGNANDIOL						
Urine ¹	.					
Range	n	2-6	3-10	0-4	0	0
Average	0	4	6	Traces	0	0

¹ In mouse uterine-weight units per 24 hours.

^{2a} In mouse ovarian-weight units per 100 cc.

³ In international units per 24 hours, assay made on castrate mice using vaginal smear method (1 m.u. approximately equivalent to 5 I.U.).

² In mouse units per 100 cc, complying with technic of Fluhmann.

³ Mg. of sodium pregnandiol glucuronide per 24 hours.

⁴ Occasionally even higher peaks may be present, or occasionally more than one peak in one cycle.

⁵ Two peaks in estrogen excretion are generally observed, one at the time of ovulation and another four or five days premenstrual.

of pregnancy. Chorionic gonadotrophin rises rapidly (blood and urine) during the early weeks of gestation to reach a very high peak, usually between the sixth and twelfth weeks, falling off subsequently to more moderate levels at about the twentieth week, after which time the gonadotrophin concentration remains fairly constant for the remainder of the period of gestation (Figs. 23 and 24, Table 19). The early, rapid rise in gonadotrophic hormone has furnished the basis for a number of popular

demonstrated in extracts of the urine of sexually mature males. The hormone appears to be chiefly of the follicle-stimulating type. Some degree of daily variation in gonadotrophin excretion has been noted, but there is no definite cyclic periodicity, such as occurs in women. The quantity of gonadotrophin in the blood of males is usually too small to be demonstrated by ordinary methods of clinical assay.

Pregnancy. ^{4,11a,27,29,31} The normal values for gonadotrophic hormone in the blood and urine during pregnancy are indicated in Figs. 23 and 24 and Table 19. It will be noted that the concentration of gonadotrophins in the serum and in the twenty-four-hour urine specimen are of approximately the same magnitude, although the urine exhibits greater day to day fluctuations.

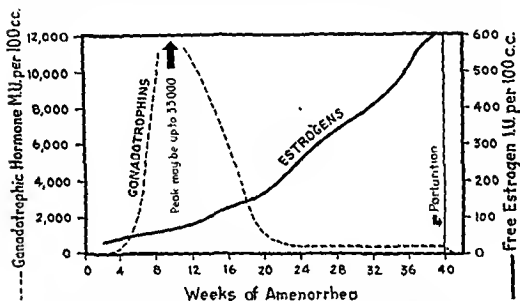


Fig. 23.—Sex hormones in the blood during normal pregnancy.

As early as ten days after conception, an increase in gonadotrophins may be demonstrated in the blood and urine, and within ten days after the first missed period there is a sufficient concentration in the urine to give a positive "pregnancy test." By the sixth week of gestation values as high as 10,000 mouse ovarian units per 100 cc. of blood serum (and urine) are commonly present and often a transient peak, up to 30,000 units, may be encountered between the sixth and twelfth weeks of gestation.

After the twentieth week the hormone level falls, and during the last half of the period of gestation the values remain quite constant, at from 300 to 500 mouse ovarian units per 100 cc. After delivery of the placenta, gonadotrophins fall rapidly, and within a week they have usually reached nonpregnant levels,

Normal Gonadotrophin Values

Childhood.^{1,4,23} It is usually not possible to detect gonadotrophins in the urine before eleven years of age. In girls, it is often possible to detect small amounts of follicle-stimulating hormone as early as a year before the onset of the first menstruation, while in boys a positive response usually cannot be obtained before the age of twelve to thirteen years.

TABLE 19
HORMONE VALUES IN PREGNANCY

Weeks of amenorrhea	Serum estrogens I.U./100 cc.*		Serum gonadotrophins m.u./100 cc.†		Pregnandiol (urine) mg./24 hrs.	
	Range	Average	Range	Average	Range	Average
2	15- 80	30			2- 10	6
4	15- 100	40	50- 1,000	200	5- 15	10
8	30- 125	60	500-33,000	10,000	5- 15	10
12	45- 165	80	1,000-33,000	10,000	8- 20	12
16	80- 200	125	1,000-33,000	6,000	8- 30	20
20	165- 250	165	330-10,000	1,000	16- 32	25
24	165- 330	250	200- 500	330	20- 60	40
28	100- 400	330	200- 500	330	35- 80	50
32	250- 600	400	200- 500	330	40- 80	60
36	330- 750	500	200- 500	330	50-100	68
40	330-1500	600	200- 500	330	50-120	70

* In this assay 1 m.u. approximately equivalent to 5 I.U.

† Mouse ovarian units (macroscopic).

Female Reproductive Period.^{9,17,20,23,29} Variable amounts of gonadotrophic hormone are excreted in the urine throughout most of the menstrual cycle in normal adult females. During the first week of the cycle the amount of hormone is usually quite small (traces to 8 mouse uterine-weight units). As the midcycle is approached, the titer suddenly rises and may reach a peak of 30 mouse uterine-weight units or more, falling suddenly subsequently. More than one such peak may appear during some cycles; it is not known whether this indicates the occurrence of more than one ovulation. Since such increases in excretion may occur quite suddenly, it is necessary to make assays at frequent intervals if this phenomenon is to be used as evidence of normal pituitary stimulation of ovulation. Gonadotrophic hormone may usually be demonstrated in whole blood or serum at the mid-cycle or during peaks of gonadotrophin excretion.¹⁵

Male Reproductive Period.^{18,20,22,29,40} Moderate amounts of gonadotrophic hormone (8-20 mouse units) may be regularly

chorionepithelioma and chorionic tumors of the testes, frequently give positive results (p. 535). It is frequently stated that positive tests are sometimes obtained in pituitary tumors and hyperthyroidism, but this is rare in our experience.

A false negative test for pregnancy may be obtained if the test is performed too early, within ten days after the first missed period, or if intrauterine fetal death has occurred. However, in the latter instance the pregnancy test may remain positive for several days to several weeks after death of the fetus.

Abnormal Gonadotrophin Values

The quantity of gonadotrophin in the blood and urine may be influenced by several factors. Increased amounts of hormone may be produced by the pituitary gland in various states of functional overactivity in which gonadotrophins may be produced (a) alone, in response to a specific stimulus, or (b) together with other pituitary hormones, when there is a state of general hyperactivity of the gland. Certain pituitary tumors may be accompanied by excessive gonadotrophin production, but this is by no means consistent or marked. Tumors of chorionic tissue, however, usually give rise to enormous increases in gonadotrophin of the luteinizing type. It is questionable whether any other type of tumor can produce gonadotrophic hormone, although this possibility has arisen in the case of other embryonal tumors, particularly of the testes.

The quantity of hormone in the blood and urine may be diminished in the presence of impaired pituitary activity, as a result of (a) a tumor involving the anterior hypophysis or neighboring structures, (b) states of functional panhypopituitarism, (c) specific functional deficiency in production of gonadotrophic hormones or (d) suppression of gonadotrophin formation by excessive amounts of gonadal hormones (estrogens, androgens). During pregnancy, there is a fall in gonadotrophins following expulsion of the placenta, in functional abnormalities of the placenta and in intra-uterine fetal death.

Increase in Gonadotrophic Hormones. (1) *Pituitary Tumors.*² Tumors of the anterior hypophysis seldom produce excessive amounts of gonadotrophic hormones, and rarely is the increase marked; normal or subnormal values are usually obtained. Since these hormones are believed to be produced by the pituitary basophils, one would expect to find increased amounts in cases of pituitary basophilism (Cushing's syndrome); however, this occurs only sporadically, and usually early in the course of this disease. Later, gonadotrophins may not be demonstrable in the blood or urine.

although occasionally titers sufficient to give a positive "pregnancy test" may persist for several weeks, particularly if fragments of placenta have been retained.

Pregnancy Tests. The demonstration of increased amounts of gonadotrophin in the blood or urine constitutes the basis for the reliable tests for pregnancy. The two procedures that have stood the test of time are the Friedman and the Aschheim-Zondek tests, both of which are highly reliable when done properly. The South African frog (*Xenopus laevis*) also appears to be a reliable test animal,²¹ and has the advantage of giving rapid results, a positive response consisting in the extrusion of eggs within four to twelve hours after injection of serum or urine

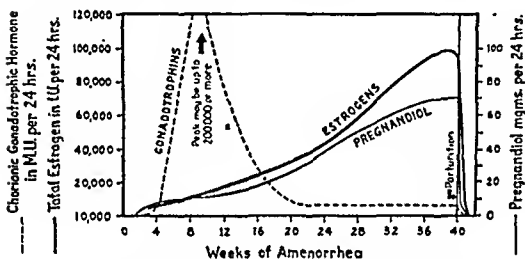


Fig. 24.—Sex hormones in the urine during normal pregnancy.

or their extracts. The South African frog has the added advantage of responding only to chorionic gonadotrophin, making the test more specific, but more data must be gathered before this method can be assumed to be as accurate as the Friedman or Aschheim-Zondek reactions. Another test for the rapid diagnosis of pregnancy is based upon the hyperemia produced in the ovaries of immature white rats in two to six hours following injection of pregnancy urine.²²

Since these "pregnancy tests" indicate merely the presence of large amounts of gonadotrophin, it is apparent that they are not specifically diagnostic of pregnancy. Fortunately, there are but few other conditions in which gonadotrophic hormone is produced in amounts sufficient to give a positive reaction. Occasionally menopausal women will produce a sufficient amount of hormone to give a weakly positive reaction. If the test is repeated, using a smaller quantity of blood or urine, such false positives can usually be distinguished from pregnancy reactions. Chorionic tumors of all kinds, including hydatidiform

trophin is entirely of the luteinizing type; and, moreover, differs from pituitary luteinizing hormone (p. 525), although this distinction is not readily demonstrable by the usual methods of assay.

In normal pregnancy the hormone is demonstrable within ten days after the first missed period, in amounts sufficient to give positive biologic tests with small amounts of blood or urine, increasing enormously within six weeks (p. 531).

Hydatidiform mole, being a tumor of chorionic tissue, usually produces an even greater amount of gonadotrophic hormone than is observed in normal pregnancy (but no estrogen nor progesterone), up to several million mouse units per twenty-four hours in the urine, or 100,000 units per 100 cc. of serum. In cases in which such striking increases are not obtained, the diagnosis cannot be made readily by laboratory methods, because this condition usually occurs at about the end of the first trimester of pregnancy, at which time very high values are encountered normally.

Very high values are obtained also in chorionepithelioma, which is a condition of malignant degeneration of the chorion. Inasmuch as this condition follows expulsion of a mole, miscarriage or normal pregnancy, the finding of progressively increasing amounts of gonadotrophin under such circumstances is highly significant, since the hormone titer should normally be diminishing rapidly. It should be emphasized, however, that a positive "pregnancy test" is not in itself diagnostically significant in this connection, since retained pieces of placental tissue or a missed abortion may occasionally produce a positive reaction weeks or even months following delivery.

Increased quantities of gonadotrophins have been observed in the blood and urine in preeclampsia and eclampsia,^{27,34} often associated with decreased titers of estrogen and pregnandiol.^{3,27,34,38} In our experience, these findings are obtained in the majority, but not in all cases, nor does the severity of the toxemia necessarily parallel the increase in gonadotrophin. A similar increase occurs at times in some diabetic women during the last half of pregnancy.⁴³ The significance of these observations is not known.

(4) *Tumors of the Testicle.* Certain malignant tumors of the testicle produce markedly increased amounts of gonadotrophic hormone,^{12,13} often enough to give a positive "pregnancy test." Values up to 300 mouse units per 100 cc. of serum (or up to 10,000 units per twenty-four hours' urine) are not rare. In the majority of instances these tumors are believed to be chorionepitheliomas, but because of existing difficulties in histologic

(2) *Functional Hyperactivity of the Pituitary.*²² The production of increased amounts of gonadotrophic hormones by the pituitary usually results from increased functional demand for gonadal stimulation. At puberty, increased amounts are required to stimulate ovarian or testicular activity, but this increase is promptly checked by the consequent production of estrogens and androgens (push-pull theory), except in the presence of a primary ovarian or testicular deficiency, in which case increased gonadotrophic excretion (up to 200 mouse units per twenty-four hours) may persist for many years. Such deficiency of the gonads may result from agenesis, orchitis or oophoritis (particularly after mumps), failure of descent of the testes after puberty, cystic disease of the ovaries, trauma and castration.^{1,18,21,27}

With the approach of the menopause, increasing amounts of gonadotrophins are produced in an effort to maintain function of the failing ovaries. This increased hypophyseal activity may persist for many years in some women, and is believed to be responsible for many of the symptoms of the menopause, particularly the hot flashes and general vasomotor instability. There is reason to believe that in some women there may be an associated overproduction of other pituitary factors, particularly the thyrotrophic and adrenocorticotrophic hormones. Increased amounts of gonadotrophins may appear within a week after removal of the ovaries and may remain at more than 100 mouse units for periods varying from six months to more than thirty years. The increase that follows "castration" by x-ray or radium is usually more gradual. Some women pass through the menopausal period with very little increase in gonadotrophins, and in occasional instances the hormone may fall to very low levels. In such cases the menopausal syndrome is likely to be atypical.

In the male, the increase in gonadotrophins at the time of the "climacteric" is not as consistent nor as marked as in the female.

Various menstrual disorders are frequently accompanied by increased excretion of gonadotrophins.^{15,16,29} In amenorrheic women, this indicates a primary ovarian deficiency. An increase is present in some cases of functional bleeding, particularly at puberty and at the menopause, being usually indicative of primary ovarian dysfunction. The persistent stimulation of abnormal ovaries by increased amounts of gonadotrophin may eventuate in cystic disease of the ovaries.

(3) *Increased Production by Chorionic Tissue.* By far the largest amounts of gonadotrophic hormone are encountered in the presence of growing chorionic tissue. The chorionic gonado-

It must be emphasized that not all malignant tumors of the testis are associated with increased gonadotrophin values. Moreover, early in the course of development of those tumors that do produce gonadotrophin, the values may not be sufficiently high to give a positive reaction with the ordinary qualitative "pregnancy tests," and quantitative assay of the urine or blood may yield helpful information.

Decrease in Gonadotrophic Hormone. (1) *Primary Pituitary Hypofunction.* The blood and urine gonadotrophins are diminished or absent in panhypopituitarism, as are all anterior pituitary hormones. They are absent in Simmonds' disease (pituitary cachexia), with consequent atrophy and functional failure of the gonads. Chromophobe tumors of the hypophysis are almost invariably accompanied by a diminution in gonadotrophic hormones. Basophilic (Cushing's syndrome) and eosinophilic (acromegaly) tumors may show some increase early in the course of their development, but later are usually associated with diminution in or absence of gonadotrophins.

The fact should be emphasized that many patients with obesity and hypogonadism, classified clinically as "hypopituitarism" or "Froelich's syndrome," may have normal or even excessive amounts of gonadotrophin, indicating that these are not true instances of hypopituitarism, but should be classified as primary hypogonadism. There is no consistent pattern of gonadotrophin excretion in patients with simple obesity, normal, decreased or increased values having been obtained in these cases.

In the female, diminished or irregular gonadotrophin excretion is commonly observed in association with infertility and with various menstrual disturbances, including amenorrhea, menorrhagia and other types of functional bleeding. Consistent and repeated failure to demonstrate adequate amounts of gonadotrophin in the urine of such patients is the only available method of establishing pituitary insufficiency as the underlying cause of the condition. This is of the utmost importance in arriving at an etiologic diagnosis and in instituting rational therapy.

Diminished or irregular gonadotrophin excretion occurs much less frequently in the male than in the female. Diminished pituitary function is not a common cause of male infertility or impotence, which are much more often due to testicular dysfunction or to systemic causes.

Malnutrition and various metabolic disorders may be followed by amenorrhea or other evidences of gonadal failure, due in some instances to diminished gonadotrophic activity. In our

classification there is some question as to whether other embryonal tumors may not produce gonadotrophic hormones. As a general principle, the largest amounts of hormone are produced by the more embryonal types of tumor. Metastases are often accompanied by marked increase in hormone concentration, while a decrease often follows removal or irradiation of the testicular tumor.

TABLE 20
VARIATIONS IN GONADOTROPHIN VALUES

Condition	Gonadotrophin values
Panhypopituitarism Simmonds' disease	Diminished or absent
Pituitary Tumors Eosinophilic Gigantism Acromegaly Basophilic Cushing's syndrome Chromophobe	Normal or increased early Diminished later Normal or increased early Diminished later Usually diminished or absent
Hypogonadism Primary gonadal failure Castration Atrophy, etc. Secondary (normal gonads)	Increased Usually diminished, sometimes normal
Menopausal Syndrome	Usually increased
Male Climacteric	Usually increased, but not as consistently nor as markedly as in women
Testicular tumors Chorionepithelioma Other embryonal tumors Interstitial cell tumors Seminomas	Increased, often markedly Moderate increase? Usually normal
Functional Menstrual Disorders	Variable. Depends on etiology, ovarian function, etc.
Pregnancy Normal Hydatidiform mole Chorionepithelioma Intra-uterine fetal death Eclampsia and preeclampsia Diabetes Retained placental tissue Missed abortion	Enormous increase. Varies with period of gestation (Figs. 23 and 24) Usually increased Progressive increase Progressive decrease Often increased Sometimes increased late in pregnancy Delayed fall Delayed fall

to constitute the most important physiologic stimulus to normal thyroid activity. Administration of this factor prevents atrophy of the thyroid in hypophysectomized animals and induces hyperplasia of the thyroid and increased secretion of thyroid hormone in normal animals. In laboratory animals it has been found to decrease the iodine content of the gland and to increase the organic iodine content of the blood; it induces creatinuria, increased oxygen consumption and decreased hepatic glycogen, and hastens the metamorphosis of tadpoles, characteristic "thyroid" effects. However, recent studies suggest that all of the actions of the thyrotrophic principle cannot be regarded as due entirely to stimulation of formation of thyroid hormone.⁸

Although thyrotrophic hormone has not been isolated in pure form, highly purified pituitary extracts are available that contain only traces of other pituitary hormones. Thyrotrophic hormone can be assayed by its effects in inducing metamorphosis in the axolotl and frog tadpole, and in causing increase in weight and histologic evidence of hyperactivity of the thyroid gland of the immature chick or young guinea pig. The last is the most commonly employed test object.

Thyrotrophic activity has been demonstrated in extracts of normal human blood and urine, but the assay methods present technical difficulties which make their application for routine clinical purposes impracticable.⁸

Increased Thyrotrophic Activity. Thyrotrophic hormone has been found to be increased in the blood and urine of some patients with acromegaly. An increase has been observed in patients with spontaneous myxedema and following thyroidectomy in euthyroid and hyperthyroid subjects. This increase has been explained on the basis of the "push-pull" hypothesis; *i.e.*, under normal conditions, thyrotrophic hormone stimulates secretion of thyroid hormone by the thyroid gland, the latter hormone, in turn, when it attains an adequate concentration in the blood, depressing formation of thyrotrophic hormone by the hypophysis. When thyroid hormone cannot be secreted in adequate amounts, this "brake" is released, permitting excessive secretion of thyrotrophic hormone. Variable findings have been reported in obesity and in Cushing's syndrome.

Decreased Thyrotrophic Activity. The thyrotrophic activity of the urine has been found to be diminished in Simmonds' disease (pituitary cachexia). A decrease (blood and urine) has also been observed in the majority of patients with hyperthyroidism. There is some evidence that suggests that the decrease in hyperthyroidism may be due to inactivation of thyrotrophic hormone by thyroxin rather than to actual suppression of formation of the former by the pituitary.

experience, the same mechanism may operate in cases of amenorrhea due to marked emotional disturbance, although the latter may also exert its influence in some cases directly on the gonad.⁴¹

During pregnancy, a decrease in gonadotrophin titer below the normal range for that period of gestation usually indicates placental failure. In cases of intra-uterine fetal death or threatened miscarriage, the gonadotrophin content of the blood and urine usually falls progressively. It has been noted that when the serum gonadotrophin content falls below 100 mouse units per 100 cc. intra-uterine fetal death has occurred or may be expected, even though the usual pregnancy tests are still positive.⁴² The latter usually become negative in such cases after several days to several weeks. In ectopic pregnancy, the concentration of gonadotrophin is often below the level expected in normal gestation, probably because of poor implantation. The Friedman and Aschheim-Zondek tests may be negative in about 25 per cent of such cases, especially in the presence of tubal rupture or abortion.

(2) *Suppression of Gonadotrophin Production or Release.* Excessive amounts of estrogen, androgen and, possibly, progesterone, may inhibit the production or release of gonadotrophins by the pituitary. This phenomenon may be observed clinically following administration of large amounts of these hormones. It occurs also during normal pregnancy, when the pituitary gonadotrophins are suppressed by the increasing level of estrogenic hormone. The same mechanism may be responsible for the low gonadotrophin values sometimes observed in patients with adrenocortical hyperfunction accompanied by high estrogen or androgen values.

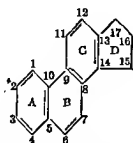
GROWTH HORMONE^{11,24}

Although growth is a complicated process which is influenced by many intrinsic and extrinsic factors, it is now generally agreed that growth is specifically influenced by a hormone elaborated by the anterior hypophysis. This growth hormone, which has been isolated in pure form, is a water-soluble substance of protein nature, produced by the eosinophilic cells of the anterior hypophysis. Growth hormone can be assayed by its ability to stimulate growth and nitrogen retention in hypophysectomized rats, but there is as yet no clinically available method for its demonstration in the blood or urine.

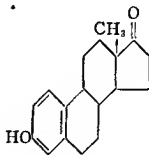
THYROTROPIC HORMONE⁸

The anterior pituitary (probably the eosinophilic cells) secretes a principle, the thyrotrophic hormone, which is believed

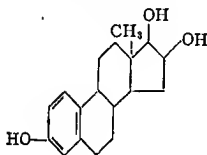
estrin) has hydroxyl groups at positions 3 and 17. Estriol (trihydroxyestrin; theelol) has three hydroxyl groups, at positions 3, 16 and 17. These estrogens are soluble in ether, alcohol, acetone and many oils, but not in petroleum ether. They are practically insoluble in water, but are quite soluble in aqueous alkali.



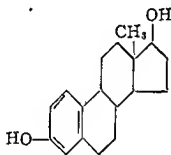
Cyclopentenophenanthrene
Structure



Estrone



Estriol



Estradiol

Fig. 25.

Sources.⁸ Estradiol has been isolated from the ovaries of the sow, the urine of pregnant women and pregnant mares, human placentas and the testes of stallions. Estrone has been isolated from the urine of pregnant women, pregnant mares, men and stallions, from the human placenta, testes of the stallion, beef adrenals and palm kernels. Estriol has been isolated from the urine of pregnant women, from human placentas and from pussy willow. The following estrogens have also been isolated from the urine of pregnant mares: equilin, hippulin, equilenin, and dihydroequilenin.

Actions. A hormone is said to be estrogenic if it induces, in a castrate animal, those changes which result from ovarian follicular activity. These consist in (a) growth of the epithelium of the genital tract, particularly the endometrium and vagina, (b) growth of the myometrium and (c) development of the duct system of the breast and the nipple. In addition, the natural estrogens are known to exert many other effects, such as retention of sodium, chloride and water, mobilization of calcium in

LACTOGENIC HORMONE^{10,11}

The anterior hypophysis secretes a specific lactation hormone which has been designated the lactogenic hormone or prolactin. There is evidence that prolactin is formed in the eosinophilic cells. It was the first of the anterior pituitary hormones to be isolated in pure form and is of protein nature, soluble in water and insoluble in fat solvents.

The lactogenic hormone is believed to be responsible for lactation after the duct-alveolar system of the breast has been prepared by the ovarian hormones (estrogen and progesterone). It is apparently formed during pregnancy, but its escape from the pituitary gland is believed to be "blocked" by the high titer of estrogens. In the postpartum period the estrogens diminish rapidly, releasing lactogenic hormone, which then stimulates lactation in the prepared breast.

Lactogenic hormone is usually assayed by means of its stimulating effect upon the crop-sacs of pigeons. It has been demonstrated in the urine of postpartum women and also in the urine of normally menstruating women, normal men and newborn infants. As yet, these observations have had no significant clinical application.

CORTICOTROPHIC HORMONE^{1,21}

The corticotrophic (adrenotrophic; adrenocorticotrophic) hormone of the anterior hypophysis is responsible for maintaining the normal structure and function of the adrenal cortex. It is protein in nature, and water-soluble, and has been isolated in pure form.²² Bioassay of this hormone presents many difficulties, the most reliable method consisting in determining the weight increase produced in the adrenal cortex of hypophysectomized rats. Although no entirely satisfactory method is available for the demonstration of corticotrophic hormone in the blood or urine, an increase has been observed in the blood of patients with Cushing's syndrome.²⁶

ESTROGENS^{1,7,9,23}

Properties of Natural Estrogens. All of the naturally occurring sex hormones, including estrogens, progesterone and androgens, are sterols, related chemically to cholesterol, bile acids, vitamin D, cardiac glycosides, and certain toad poisons and hemolytic saponins, all built about a cyclopentenophenanthrene nucleus (Fig. 25). In estrone (ketohydroxyestrin; theelin), the first ring (A) is unsaturated, with a hydroxyl group at carbon atom 3 and oxygen at carbon atom 17. Estradiol (dihydroxy-

estrin) has hydroxyl groups at positions 3 and 17. Estriol (trihydroxyestrin; theelol) has three hydroxyl groups, at positions 3, 16 and 17. These estrogens are soluble in ether, alcohol, acetone and many oils, but not in petroleum ether. They are practically insoluble in water, but are quite soluble in aqueous alkali.

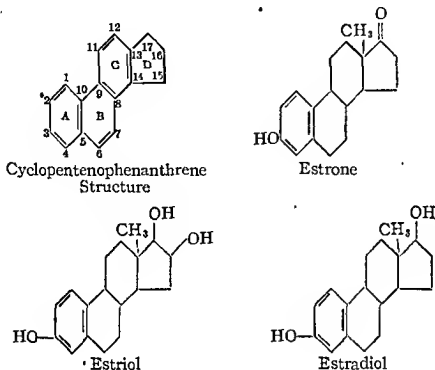


Fig. 25.

Sources.⁸ Estradiol has been isolated from the ovaries of the sow, the urine of pregnant women and pregnant mares, human placentas and the testes of stallions. Estrone has been isolated from the urine of pregnant women, pregnant mares, men and stallions, from the human placenta, testes of the stallion, beef adrenals and palm kernels. Estriol has been isolated from the urine of pregnant women, from human placentas and from pussy willow. The following estrogens have also been isolated from the urine of pregnant mares: equilin, hippulin, equilenin, and dihydroequilenin.

Actions. A hormone is said to be estrogenic if it induces, in a castrate animal, those changes which result from ovarian follicular activity. These consist in (a) growth of the epithelium of the genital tract, particularly the endometrium and vagina, (b) growth of the myometrium and (c) development of the duct system of the breast and the nipple. In addition, the natural estrogens are known to exert many other effects, such as retention of sodium, chloride and water, mobilization of calcium in

certain species, and suppression of certain anterior pituitary hormones, particularly the gonadotrophins and prolactin. In the male, estrogens may produce the physiologic effects of castration, probably by inhibiting pituitary gonadotrophic activity.

NORMAL PHYSIOLOGY OF ESTROGENS IN HUMANS

Estrogen is produced by various cells of the ovary, including the follicular epithelium, theca cells, granulosa cells and lutein cells, under stimulation by the gonadotrophic hormones. The hormone secreted by the ovary is probably alpha-estradiol. Estrogen production by the ovary begins several years before the menarche, and is largely responsible for the manifestations of puberty, particularly the development of the genital tract, external genitalia and breasts, and the appearance of pubic and axillary hair. The quantity of hormone gradually increases until the menarche, and is produced in a cyclic rhythm after menstrual function is well established (Fig. 22 and Table 18).

During the first half of the menstrual cycle it is secreted by the growing follicle in increasing amounts, reaching a maximum at the time of ovulation, when the follicle ruptures, releasing follicular fluid rich in estrogen. A brief fall occurs in the post-ovulatory stage, followed by a second rise as the corpus luteum becomes active functionally and produces considerable amounts of estrogen in addition to its specific hormone, progesterone. This second peak in estrogen activity occurs about four to five days before the next menstrual period, which is, in fact, induced by the rapid fall in the hormone concentration in the immediate premenstrual phase.

The ovarian estrogen (alpha-estradiol) enters the systemic circulation and reaches the liver. It is generally believed that it is largely permanently and rapidly inactivated in this organ;²¹ there is evidence,^{3,4} however, that it is excreted in the bile in active form, probably as a mixture of estrogens, subsequently undergoing an enterohepatic circulation during which the bulk of the hormone is probably gradually metabolized in the liver, small amounts being released into the systemic circulation for many days. Only about 5-10 per cent of the estrogenic activity of exogenous and probably also endogenous estrogens can be recovered in the urine. The estrogens in the urine consist chiefly of estrone and estriol, with only traces of estradiol. These are present chiefly in a conjugated state, as glucuronides, from which they can be freed by acid hydrolysis. There is evidence that the uterus aids in the conversion of estrone to estriol, and that progesterone tends to prevent excessive destruction of estrogens.^{23,25}

During the early weeks of pregnancy, estrogen continues to be formed by the corpus luteum of pregnancy, but is gradually replaced by estrogen from the placenta, which produces the hormone in large amounts, chiefly as estriol; however, it contains also considerable quantities of estrone and estradiol.^{5,26} As gestation progresses, the estrogenic content of the blood and urine increases progressively (Figs. 23 and 24 and Table 19). In the blood, the hormone is present chiefly in the free state, but as much as 25-50 per cent may be bound to the plasma proteins.²⁴ Very large quantities of estrogen are present in the urine of late pregnancy, as much as a hundred thousand international units per twenty-four hours, over 99 per cent of which may be in conjugated form, largely as estriol glucuronidate. With the approach of term, there is a considerable increase in free estriol in the urine, at the expense of the conjugated form.⁵ This increase in free estrogen is believed to aid in sensitizing the uterus for the onset of labor. During pregnancy the estrogens serve to promote the growth of the uterus, to develop the duct system of the breasts in preparation for lactation, to cause persistence of the corpus luteum and to aid in the mechanism of labor.⁶

As the menopause approaches, estrogen production gradually diminishes. Inasmuch as small amounts are present in castrates, it is believed that some estrogenic substances are produced elsewhere in the body, probably in the adrenal cortex. Estrogenic activity is also present in the urine of men and considerable amounts in the urine and testes of stallions. It is probable that the estrogenic activity of the urine of men originates in steroids derived from the adrenal cortex and testes.

METHODS FOR DETERMINING ESTROGENS

Although an estrogenic effect may be estimated by cytologic examination of vaginal smears or histologic study of endometrial tissue obtained by biopsy or curettage, the only direct method for determining the hormone quantitatively consists in the biologic assay of blood or urine. Some modification of the Allen-Doisy procedure is usually employed, in which the material or extract is injected into castrated adult mice or rats and various degrees of vaginal estrus are determined. Such tests are capable of detecting very small amounts of estrogen. It must be emphasized that minor alterations in technic may result in wide degrees of variation, so that findings from different laboratories may not be comparable; each laboratory must interpret findings on the basis of its own standards. Confusion frequently arises also from the fact that estrogen activity may be expressed in terms

of mouse units (m.u.), rat units (r.u.) or international units (I.U.). Moreover, these units cannot be accurately interconverted if a mixture of estrogens is present. For estrone, which is the chief estrogen in the urine of nonpregnant subjects, 1 rat unit is usually equivalent to about 10 mouse units, while one mouse unit may equal 1-5 international units (I.U.), depending upon the method of assay. The potency of estradiol may be 2-12 times that of estrone, depending upon the method of assay, while that of estrone may be 1-266 times that of estriol. The following international standards have been established on the basis of weight of the crystalline hormones: (1) Estrone: One international unit (I.U.) is equivalent to 0.0001 mg. (0.1 gamma); (2) Estradiol benzoate: One international benzoate unit (I.B.U.) is equivalent to 0.0001 mg. (0.1 gamma).

The quantity of estrogen in the blood of nonpregnant women is very small, but it can be detected during certain phases of the menstrual cycle by injection of either whole serum (Fluhmann)¹⁰ or extracts of blood (Frank and Goldberger).¹² During pregnancy, the quantity of estrogen in the blood is so great that it can be assayed easily. Estrogens are present in the urine chiefly in a conjugated state and must therefore be freed by acid hydrolysis, extracted with a steroid solvent and partially purified before being assayed.

A number of colorimetric methods are available for the quantitative determination of estrogens. At the present time, these are useful only when relatively large amounts of hormone are present, as in the urine during pregnancy.

NORMAL ESTROGEN VALUES

Childhood. Even very young children excrete small amounts of estrogen in the urine.¹⁹ After eight years of age, there is an increase in the urinary estrogen of girls, and some cyclic variation may occur as early as one and a half years before the menarche. The normal range of estrogen values is indicated in Table 18.

Reproductive Period. (Fig. 22 and Table 18). There is a cyclic variation in estrogens in the normally menstruating female,^{10,14,27} from puberty to the menopause. The daily urinary excretion increases from a low level at the onset of the flow to a maximum at the midperiod, generally in association with the peak of gonadotrophin excretion. It then diminishes slightly, but rises again during the period of increased corpus luteum function and reaches a second peak in the premenstrual phase, to be followed by a rapid drop immediately preceding the flow. The first peak may occasionally be higher than the second, but

we have found the opposite to occur more commonly. There is considerable variation in the shape and height of the curves in different individuals and from month to month in the same individual.

Similar findings may be obtained with blood serum (Fluhmann technic), while the premenstrual peak can usually be demonstrated by the Frank-Goldberger method. For most purposes, blood estrogen assays are not as reliable as urinary findings, since only a small amount of hormone is present in the quantity of blood that can be conveniently withdrawn. In some instances, however, determinations of both blood and urine estrogens are useful (Figs. 23, 24 and Table 19). During pregnancy, direct assays of the blood may be made conveniently and accurately, because of the high estrogen levels.

Climacteric. The menopausal period is characterized by a decrease in estrogens and an increase in gonadotrophins. Following the menopause, the urine estrogens usually fall to less than 13 m.u. per twenty-four hours (65 I.U.).

Pregnancy. The estrogen content of the blood and urine increases gradually and progressively to a maximum at term, at which time very high titers are obtained (Figs. 23 and 24; Table 19).

In the blood, the free estrogen level during the first eight weeks of pregnancy ranges from 45 to 80 I.U. per 100 cc., which is still within the normal, nonpregnant range. By the twelfth week it has risen to more than 125 I.U. per 100 cc., a level definitely higher than is likely to be encountered in any state other than pregnancy. Certain pregnancy tests have been based upon this phenomenon, but the much earlier and much greater increase in gonadotrophins is more satisfactory for this purpose. After the twentieth week, the blood estrogen curve rises somewhat more rapidly to a maximum, usually of 600-1500 I.U., but occasionally as high as 2500 I.U. or more per 100 cc. Prolonged acid hydrolysis may result in values 25-50 per cent higher than those for free estrogen, the additional estrogen being in combined form. The estrogen level begins to fall in a few hours after delivery and reaches nonpregnant levels within three to four days.

The total estrogen content of the urine also increases rapidly and progressively throughout pregnancy, to reach a maximum at term. Up to the eighth week, values of 200-1000 I.U. per twenty-four hours are obtained commonly. At times the total estrogen excretion may range from 15,000 to 100,000 I.U. or more per twenty-four hours, often with wide daily fluctuations. The estrogens present in pregnancy urine are

estradiol, estrone and estriol. These are present in both free and conjugated forms, but more than 90 per cent is generally conjugated. The relative quantities of the various estrogens vary widely as pregnancy progresses. It has been found that the ratio between estrone and estriol changes from about 1:2 in the second month to about 1:15 at nine months.⁶ Estradiol is excreted at a fairly uniform rate throughout pregnancy, averaging about 0.13 mg. daily. Immediately preceding parturition the bulk of the urine estrogen, chiefly estriol, changes rather suddenly from the conjugated to the free state, and there is a fall in the total excretion of estrogens.²⁷

INCREASED ESTROGEN VALUES

Increased Production. In true *precocious menarche*, gonadotrophin and estrogen values similar to those found in normally menstruating women may occur in association with normal manifestations of puberty.¹⁸ Such cases must be differentiated from precocious puberty due to granulosa-cell tumor or other neoplasms.

Very few tumors produce increased amounts of estrogen, certainly not quantities comparable to those formed late in pregnancy. *Granulosa-cell tumors* of the ovary are usually accompanied by a moderate increase in the estrogen content of the blood and urine.^{15, 17, 20} This increase can often be detected in cases occurring in children before puberty and in women after the menopause. It is also evidenced by obvious estrogenic effects in the patient. The total urine estrogen may range from 50 to 400 m.u. per twenty-four hours. The diagnosis of granulosa-cell tumor is more difficult in women during the reproductive period because the urine estrogens may be within normal limits and the clinical estrogen effects are not obvious.

Increased amounts of estrogen may be found in some instances of *tumor or hyperplasia of the adrenal cortex*. Very high values (more than 500 I.U. per twenty-four hours) have been regarded by some as characteristic of adrenocortical carcinoma.¹² However, this is by no means true of all cases of cortical carcinoma, so that negative findings cannot be regarded as significant in the diagnosis of this condition.

A moderate increase in estrogen excretion has occasionally been observed in cases of *testicular tumors* or chorionepithelioma origin, but normal findings are the rule in such cases.

Functional ovarian disorders are seldom accompanied by marked increase in the blood or twenty-four-hour urine estrogens. It is not unusual, however, to find a persistently high plateau of estrogen excretion, which may result in a state of

hyperestrogenism. The monthly output of estrogens under such circumstances may be two to five times normal. This may be associated with various types of functional bleeding or amenorrhea, depending upon the pattern of estrogen excretion and the responsiveness of the uterus. Not uncommonly, it is associated with amenorrhea followed by prolonged periods of bleeding and a hyperplastic endometrium ("metropathia hemorrhagica").

Decreased Destruction. A considerable increase in the free estrogen content of the urine, with or without an increase in total estrogen excretion, has been observed in patients with *hepatic functional impairment*.¹⁶ This has been attributed to diminished destruction or inactivation (or excretion) by the liver. Such patients often exhibit clinical manifestations of hyperestrogenism, such as gynecomastia and testicular atrophy in the male, and menstrual disorders in the female. There is some evidence that vitamin B deficiency² may interfere with hepatic inactivation of estrogens and thus contribute to the pathogenesis of various disorders such as menstrual dysfunction, chronic cystic mastitis, premenstrual tension, and so on.

DECREASED ESTROGEN VALUES

In women during the reproductive period, decreased estrogen values in the blood and urine may result either from (a) primary pituitary deficiency, with inadequate gonadotrophic stimulation of the ovaries, or from (b) a primary ovarian defect, despite normal or even excessive gonadotrophic stimulation. The distinction between primary and secondary ovarian (estrogen) deficiency can usually be made with certainty only by gonadotrophin assays.

Decreased estrogen values are found in Simmonds' disease and milder forms of panhypopituitarism, and in other conditions associated with diminished gonadotrophin production (p. 537). Primary estrogen deficiency in young girls may result in a state of eunuchoidism, sexual infantilism, amenorrhea or hypomenorrhea. Relative estrogen deficiency or irregular estrogen production later in the reproductive period may result in a variety of disturbances, including secondary amenorrhea, hypomenorrhea, various types of functional bleeding or sterility. A premature menopausal syndrome may develop if this ovarian deficiency persists. In patients with ovarian cysts and other benign lesions of the ovary, estrogen excretion may vary considerably, but is usually low.

Threatened miscarriage early in pregnancy is accompanied by marked reduction in estrogen and pregnandiol (p. 550). A marked fall in the estrogen level occurs regularly following death

of the fetus at any stage of pregnancy.¹¹ A moderate decrease in estrogen in the blood and urine has been observed in many patients with preclampsia and eclampsia and also frequently in diabetic pregnant women.^{24,25,29,30} The latter condition (diabetes-pregnancy) is also accompanied often by a diminution in progesterone, which is believed by some to be responsible for the disturbance in estrogen metabolism. Fractionation of the estrogens in toxemic patients has shown that the estrone fraction drops to very low values or disappears entirely; the estriol fraction decreases considerably and the estradiol fraction increases.

Estrogen excretion is diminished in the male castrate, indicating that a portion of the estrogen in normal male urine is of testicular origin.

PROGESTERONE^{12,14}

Progesterone is a steroid hormone secreted by the corpus luteum, which has the specific function of preparing the uterus for the reception and nourishment of the embryo. It is closely related chemically to the other sex hormones and to the bile acids.

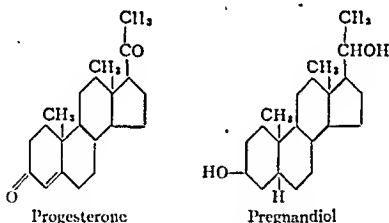


Fig. 26.

Progesterone was first isolated in crude form from the ovaries of swine by Corner and Allen⁹ and was later purified by other workers; it can now be produced synthetically. It is responsible for the secretory changes that characterize the typical premenstrual endometrium and also serves to diminish spontaneous contractions of the uterus.^{1,16} Progesterone prevents excessive destruction of estrogen and facilitates conversion of estrone to estriol in the rabbit and human. Like certain other steroid hormones, it favors retention of sodium, chloride and water in the tissues.⁸ It is believed that progesterone is metabolized and conjugated in the liver to pregnandiol glycuronide, being

excreted in the urine as sodium-pregnandiol glycuronide, a water-soluble substance which can be precipitated by acetone.¹⁹ The amount of pregnandiol recovered from the urine is utilized as an index of corpus luteum function (progesterone production).

During the first trimester of pregnancy, it is believed that progesterone is made chiefly by the corpus luteum of pregnancy, but that thereafter the placenta takes over this function. Progesterone is produced in increasing amounts as pregnancy progresses. There is reason to believe that the adrenal cortex can produce pregnandiol or pregnandiol-like substances under certain abnormal circumstances.

METHODS OF ASSAY

Progesterone may be assayed by the Corner-Allen method,¹ in which a progestational effect is produced in the endometrium of castrated rabbits. Since only minute amounts of the hormone are present in the blood, even in pregnancy, a more sensitive method has been devised, in which the material to be assayed is introduced directly into the rabbit uterus and an endometrial response is obtained with relatively minute amounts of progesterone.¹³ More commonly, progesterone is determined indirectly by the quantitative determination of pregnandiol excretion in the urine. This is usually done by gravimetric methods;^{2,19} a colorimetric method for pregnandiol has also been described.¹⁸

NORMAL PREGNANDIOL VALUES

Pregnandiol is not found in the urine of normal children or men. In the normally menstruating woman, pregnandiol appears quite suddenly in the urine about twelve to thirteen days before the onset of menstruation, *i.e.*, within a day or two after ovulation. The amount excreted daily in the urine varies, but usually ranges from 3 to 8 mg., occasionally reaching values as high as 11 or 12 mg. The total quantity excreted in the urine during a luteal phase may range from 20 to 80 mg. It usually reaches a peak about the twenty-first day of the menstrual cycle and then drops abruptly to zero, two to three days before the onset of bleeding. The curve of pregnandiol excretion thus closely parallels the development, activity and regression of the corpus luteum.¹⁰ (Fig. 22; Table 18.)

If pregnancy occurs, the pregnandiol excretion does not diminish, but increases slightly until the tenth or twelfth week, and then continues to increase further to reach a peak of 60-100 mg. daily at or near term. At the time of onset of labor the rate of excretion may diminish,⁴ this drop being regarded as a factor

in the mechanism of onset of labor (Fig. 24; Table 19). It has been suggested that the increase in excretion of pregnandiol early in pregnancy may serve as a basis for a satisfactory test for pregnancy.¹²

INCREASED PREGNANDIOL VALUES

An increase in pregnandiol or pregnandiol-like substances may occur in cases of adrenal cortical hyperfunction, as in Cushing's syndrome, the adreno-genital syndrome or tumors of the adrenal cortex not accompanied by clinical manifestations of these disorders, in both males and females.^{11,18,20} Values ranging from 2 to 25 mg. per twenty-four hours have been obtained in such cases. It is believed that in these conditions the pregnandiol is probably an excretion product of steroids originating in the adrenal cortex.

DIMINISHED PREGNANDIOL VALUES

Menstrual Disorders and Sterility. Absence of pregnandiol in the urine during the two weeks preceding menstruation is indicative of failure of ovulation and absence of a functionally active corpus luteum. In such instances the estrogen values are usually also diminished. There is still some question as to whether a normal pregnandiol excretion is conclusive evidence of the occurrence of ovulation. It should be pointed out that too much significance should not be attached to a single low value, because of the normally wide daily fluctuations. Furthermore, in studying patients with sterility in whom some defect in ovulation is suspected, it is well to correlate the pregnandiol findings with data obtained by endometrial biopsy, vaginal smears, etc. Absence of pregnandiol may be associated with anovulatory sterility, amenorrhea, and various types of functional bleeding. Patients with dysmenorrhea do not, as a rule, exhibit abnormal pregnandiol values, nor is there any specific relationship between pregnandiol and premenstrual tension, cystic mastitis or various premenstrual disturbances.

Threatened and Habitual Abortion. The great majority of spontaneous abortions occur from the eighth to the twelfth weeks, during the period when the placenta is believed to be assuming the function of the corpus luteum of pregnancy. Inasmuch as the production of normal amounts of progesterone (and estrogen) is thought to be essential for the continuation of gestation, it is suspected that premature failure of the corpus luteum of pregnancy or inadequate placental secretion of progesterone may be the etiologic factor in many spontaneous

abortions. Low pregnandiol and estrogen values have been found in patients who abort habitually during this period.⁷

Intra-uterine Fetal Death. Diminishing pregnandiol titers are found regularly in the event of intra-uterine fetal death, although some days may elapse before low titers are reached.

Toxemias of Late Pregnancy. There are a number of reports indicating that preeclampsia and eclampsia are accompanied by an abnormally low twenty-four-hour excretion of pregnandiol,^{3,6,17} as well as of estrogen (p. 548). The significance of this observation is not known. It is often associated with an increase in gonadotrophin (p. 535).

Diabetes in Pregnancy. A similar pattern of low pregnandiol and estrogen and high gonadotrophin values sometimes occurs in diabetic pregnant women, and has been employed as a criterion for instituting substitution therapy with estrogens and progesterone.²¹

TESTICULAR HORMONE (ANDROGEN)^{12,14,18,20}

In addition to its spermatogenic function, the testis has an endocrine function, the production of male sex hormone, which is believed to be secreted in the form of testosterone. It is secreted by the interstitial cells of Leydig under the influence of pituitary gonadotrophic stimulation (interstitial-cell-stimulating gonadotrophin or luteinizing hormone). Male sex hormone is concerned with conditioning the mating drive and with maintaining the structure and function of the accessory reproductive organs, particularly the penis, prostate and seminal vesicles. It is also concerned with maintaining certain male characteristics, such as beard-growth and masculine hair distribution, deepening of the voice, male type of body development, etc.¹⁸ Testosterone is believed to play an important role in closure of the epiphyses in the growing male. Like the estrogens, androgens tend to inhibit the secretion or liberation of certain pituitary hormones, particularly gonadotrophins. Testosterone in high dosage favors retention of nitrogen, sodium chloride and water.

Testosterone is closely related chemically to the ovarian hormones and has been prepared from cholesterol; it has been obtained in crystalline form from testicular tissue. It probably undergoes metabolic changes in the liver, as do the estrogens (p. 542), and appears in the urine as a number of related steroid substances, some of which have androgenic properties, particularly androsterone and dehydroandrosterone, and others which are biologically inactive.^{3,5} Androsterone is considerably less active than testosterone (one-fifth to one-tenth), whereas dehydroandrosterone is only about one-fourth as active as

androsterone.⁸ There is reason to believe that a portion (about two-thirds) of the androgenic substances that appear in the urine of males originates in the adrenal cortex.

Androgens are present in the urine of women in amounts up to three-fourths of that in the male. It is possible that in women the hormone is derived from both the ovary and the adrenal cortex, although this is still open to question. The latter is generally believed to be the source of most, if not all, of the androgen in the female.

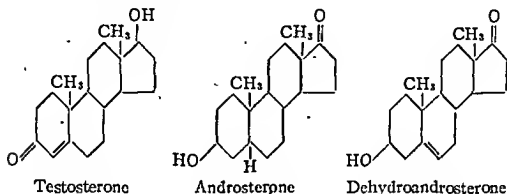


Fig. 27.

METHODS OF ASSAY

Androgens are present in the blood, but not in sufficient concentration to be readily demonstrable by methods suitable for clinical purposes.¹⁷ They are present in the urine in the form of water-soluble, biologically inactive glucuronides, and can be obtained in a free, active state by acid hydrolysis followed by extraction with steroid solvents.¹⁸ After partial purification, these extracts can be assayed by biologic or colorimetric methods. Each of these methods presents certain disadvantages. Bioassay is time-consuming, expensive and difficult. The available colorimetric methods are also somewhat complicated and, moreover, do not consistently yield results comparable to those obtained by bioassay (see 17-ketosteroids, p. 555).

Biologic Assay. The most accurate method for bioassay of androgens is based upon stimulation of comb-growth in the capon after administration of extracts of the test material by injection or by direct application (inunction) to the comb for a number of days.¹⁹ The results are expressed in terms of international units (I.U.), one international unit being equivalent to the activity of 0.1 mg. of androsterone. Methods based upon increased growth (weight) of the comb of the baby chick have also been described.⁷ Although not as accurate as the capon method, satisfactory results have been obtained by this procedure when carefully controlled.²¹ The normal values obtained

with the baby chick method are about one-seventh those obtained by the capon method, but testosterone assays about equally by these two methods. A correction factor must therefore be introduced when comparing results obtained with these procedures. Tests based upon stimulation of the prostate or seminal vesicles of the castrate rodent are not very satisfactory since other substances in the extracts may have a nonspecific augmenting effect.^{15,18}

NORMAL VALUES FOR ANDROGEN (BIOASSAY BY CAPON METHOD)

Childhood. Small amounts (up to 2 I.U. per liter) of androgenic activity¹⁵ may be demonstrated in the urine of children of both sexes. Values of 3–20 I.U. are obtained by colorimetric methods.¹⁹ A distinct rise occurs in boys soon after the age of ten years, increasing gradually subsequently to the adult male level after puberty.

Reproductive Period. Normal men excrete an average of 70 I.U. of androgen in twenty-four hours. Fluctuations from 30 to 100 I.U. occur commonly, but there is no definite cyclic variation.¹⁰

TABLE 21
ANDROGEN (BIOLOGIC ASSAY)

	Urinary excretion per 24 hours in I.U.	
	Range	Average
Children (male and female) up to 10 years.....	0.5- 20	
Young adult males	30 -100	70
Young adult females	30 -100	50

Normal women excrete almost as much androgen as men, the daily values usually ranging from 30 to 100 I.U., with average values of about 50 I.U. However, the androgen:estrogen ratio in the male is 2 to 5 times that in the female. Although there are a few reports of cyclic variations in androgen excretion in the female, the present consensus is that whereas there is considerable daily variation, this presents no definite pattern.⁹

Pregnancy. During normal pregnancy the androgen values generally fall within the range for the normal nonpregnant woman, or may be lower.¹¹

Climacteric. Diminished androgen titers are frequently found in men after the age of fifty years.¹⁶ In menopausal or post-

menopausal women, biologic androgen values may be normal or diminished.^{6,18}

ABNORMAL ANDROGEN VALUES

Consistently low values (3-40 I.U.) are obtained in male hypogonads. Inasmuch as occasional low values may be obtained in normal males, these should be checked by examination of more than one twenty-four-hour specimen. The combination of subnormal androgen and high gonadotrophin values is characteristic of primary testicular failure. This may occur in eunuchs or as a sequel of orchitis, operations interfering with the blood supply to the testes, systemic diseases, and the like. Diminution in or absence of gonadotrophic hormone in men with subnormal androgen values suggests primary pituitary hypofunction. This may occur in panhypopituitarism, pituitary tumors and true instances of Fröhlich's syndrome. A marked decrease occurs in patients with Addison's disease, particularly in women.¹⁵ Severe nutritional disturbances may also cause a decrease in androgens.¹⁶

Functional disturbances in the pituitary-gonad cycle occur much less commonly in the male than in the female. Moreover, disorders of spermatogenesis, with associated infertility, are often not accompanied by any demonstrable abnormality of male sex hormone excretion.

Excessive excretion of biologically active androgens is encountered in association with masculinizing tumors of the adrenal cortex, arrhenoblastomas of the ovary and interstitial-cell tumors of the testis.^{1,2,4,22,23} In the last condition extremely high values have been obtained, as much as the equivalent of 1000 mg. of androsterone per twenty-four hours, the hormone being present in the urine chiefly as androsterone sulfate.²² Values up to 480 I.U. per twenty-four hours have been reported in cases of adrenal carcinoma.¹³ Some cases of adrenal adenoma or cancer have failed to show an increase in androgen, although a decrease was observed after removal of the tumor.¹⁵

Increased androgen excretion occurs frequently in patients with adrenal cortical hyperplasia. The values in these cases are usually only moderately increased, but occasionally may reach as high as 500 capon units daily. A moderate increase is generally noted in Cushing's syndrome. It has been suggested that in cases of Cushing's syndrome in which the androgen values are normal, the disease is more likely to be primarily pituitary than adrenal in origin. Normal values are usually obtained in patients with simple hirsutism or virilism of constitutional origin.

17-KETOSTEROIDS^{2,8,9,10,11,18}

The term "17-ketosteroids" refers to those steroids possessing a ketone group on the 17th carbon atom. They can be measured quantitatively by the intensity of the red color that they produce with *m*-dinitrobenzene in alkaline solution (Zimmerman reaction).¹⁹

The principal 17-ketosteroids that have been isolated from normal human urine are (a) androsterone, (b) two of its isomers, isoandrosterone and etiocholanol-3 alpha-17-one, (c) dehydroisoandrosterone and (d) estrone. Estrone, a phenolic substance, can be separated from the other 17-ketosteroids by treatment with alkali. The remaining neutral 17-ketosteroids are all regarded as androstane derivatives (having 19 carbon atoms in the typical steroid skeleton) and have been loosely called the urinary "androgens."⁷ They are believed to represent the excretory transformation products of certain adrenal and testicular hormones. The quantity excreted in the male serves as an index of the combined steroid secretory activity of the adrenal cortex and the testis, and in the female chiefly of the adrenal cortex. Since some of the 17-ketosteroids are but weakly androgenic, or biologically inactive, the values obtained are not equivalent to those obtained by bioassay. In most instances, however, the colorimetric and biologic values roughly parallel each other.

The total 17-ketosteroids can be readily determined quantitatively in urine by means of the Zimmerman reaction, and also by a more specific colorimetric reaction (Pincus)⁶ which involves the development of a blue color with antimony trichloride. Since they are present in urine as esters, they must first be freed by careful hydrolysis; the free steroids are then removed by extraction with organic solvents and further purified to remove certain pigments and phenolic substances, including the urinary estrogens. If further separation from nonketonic substances is desired (not usually necessary for routine clinical purposes) this can be accomplished by treating with Girard's reagent.¹⁵

The 17-ketosteroids can be further subdivided into alpha and beta fractions, the beta-17-ketosteroids being precipitated by digitonin whereas the alpha are not.¹⁴ The alpha-17-ketosteroids normally predominate (85-95 per cent), consisting of androsterone and etiocholanol-3 alpha-17-one, while the beta-17-ketosteroids are present in only small amounts and consist of isoandrosterone and dehydroisoandrosterone. These alpha and beta compounds are alcoholic in nature; small amounts of non-alcoholic 17-ketosteroids may also be present (androstenone-17 and $\Delta_{3,5}$ androstadieneone).¹³ In normal adults, alcoholic

ketosteroids comprise 50-85 per cent and the beta-ketosteroids seldom more than 15 per cent and usually 2-3 per cent of the total (Table 22).¹³

TABLE 22
AVERAGE EXCRETION OF ALPHA, BETA AND TOTAL NEUTRAL KETOSTEROIDS BY
NORMAL SUBJECTS*

Type of subject	No. of cases	Average alpha neutral ketosteroids, mg. per 24 hours	Beta neutral ketosteroids, mg. per 24 hr.		Total neutral ketosteroids, mg. per 24 hr.	
			Average	Range	Average	Range
Children						
4 to 7.....	5	1.2	0.1	0.0-0.2	1.3	0.8-2.6
7 to 12 years....	10	3.7	0.3	0.0-0.5	4.0	1.8-5.0
12 to 15 years....	7	7.5	0.7	0.0-2.7	8.2	5.0-11.3
Women, 15+ years..	9	9.1	1.1	0.0-2.5	10.2	6.5-17.4
Men, 15+ years....	5	13.8	1.2	0.0-1.8	15.0	12.3-18.5
Women, 4 to 7 months pregnant.....	4	13.2	1.9	0.0-4.0	15.1	10.8-20.4

* Forty-nine determinations were made on 40 subjects who were hospital staff members or patients presenting no obvious evidence of endocrine disorder.

Talbot, N. B. *et al.*: N. Eng. J. Med. 223:369, 1940.

NORMAL VALUES FOR 17-KETOSTEROIDS

The values to be given apply to the neutral 17-ketosteroids as determined (a) in the crude extract, corrected for interfering chromogens, or (b) in the purified ketone fraction. The values are expressed in terms of equivalents of androsterone.

Childhood. Children under six years of age usually excrete less than 1 mg. per twenty-four hours. The values then rise slowly to about the age of eighteen, although figures within the normal adult range may be encountered after the age of twelve years (Table 22).¹¹

Adult Women. In normal adult women during the reproductive period, the 17-ketosteroid values usually range from 5 to 15 mg. per twenty-four hours. No cyclic fluctuations occur in association with the menstrual cycle.

Adult Men. In adult men, the usual average range is from 7 to 20 mg., although values up to 27 mg. daily have been regarded as normal. The higher values in men are attributable to the fact that the testes contribute to the total 17-ketosteroid output, while the ovaries do not (excluding estrone), although the possibility of a difference in adrenal cortical function in the two sexes must be considered in this connection.

It has been observed that larger quantities of 17-ketosteroids are excreted during the night than during the day,⁸ but high night titers appear to be associated with high day titers, and vice versa. Day to day fluctuations occur commonly.

Pregnancy. The 17-ketosteroid excretion (excluding estrone) in pregnancy remains within the nonpregnant range, namely, 5-15 mg. per twenty-four hours, although values as high as 20 mg. may be obtained in late pregnancy.

Climacteric. Values ranging from 3 to 18 mg. per twenty-four hours may be obtained in menopausal women. As a rule, the values are normal or diminished moderately; moderately increased values are encountered occasionally, and are thought to be indicative of increased functional activity of the adrenal cortex.³ In men, the 17-ketosteroid values usually remain within the normal adult range up to the age of fifty years. Values below 5 mg. are commonly obtained in elderly men.

DECREASED 17-KETOSTEROID VALUES

The urinary 17-ketosteroids may be subnormal in primary or secondary hypofunction of the adrenal cortex or testis (interstitial cells). Low values may also occur in many primarily nonendocrine disorders in which the relation to adrenocortical or testicular function is not apparent. Such findings must therefore be evaluated critically.²

Hypopituitarism. In panhypopituitarism, such as may be encountered in Simmonds' disease, certain brain tumors, pituitary infarction and pituitary dwarfism, the urinary 17-ketosteroids are markedly diminished or absent (0-1 mg. per twenty-four hours). The absence of 17-ketosteroids in these conditions implies lack of stimulation of the adrenal cortex and gonads by the pituitary. Absence of 17-ketosteroids may be helpful in distinguishing true panhypopituitarism from conditions which may resemble it clinically, such as anorexia nervosa, in which the 17-ketosteroids are normal or diminished (but not absent).

In milder states of pituitary deficiency, particularly if not all of the trophic hormones are affected, the 17-ketosteroids may be low-normal or moderately diminished. It is not unusual to find them only moderately diminished in patients with hypogonadism with no gonadotrophins.

Pituitary Tumors. The 17-ketosteroids have been found to be normal or moderately diminished in patients with acromegaly,² despite the fact that this condition is usually accompanied by hyperplasia of the adrenal cortex; it has therefore been suggested that the latter is compensatory in nature. Low, normal or moderately increased values may be obtained in pituitary

basophilism at various stages of the disease. The very high values that may be encountered in some cases of Cushing's syndrome associated with carcinoma of the adrenal cortex are not usually obtained in cases due to primary pituitary disease.² In chromophobe tumors of the pituitary, the 17-ketosteroids usually diminish rapidly and eventually disappear.

Male Hypogonadism. In hypogonad males, very low values (1-4 mg.) are more likely to be associated with primary pituitary hypofunction, whereas low-normal or moderately diminished values are encountered more often in primary hypogonadism. The gonadotrophins are usually high in the latter condition and low or absent in the former. These findings support the view that, in the male, only a fraction (probably about one-third) of the urinary 17-ketosteroids originates in the testis.

Female Hypogonadism. In young women with clinical evidence of ovarian deficiency, the 17-ketosteroids may vary as follows, depending upon the etiology and associated endocrine dysfunction:

- (a) Absent or low, if due to panhypopituitarism.
- (b) Moderately low if associated with various metabolic disorders, anorexia, or debilitating systemic disorders.
- (c) Normal in the majority of patients with primary ovarian failure, since the neutral 17-ketosteroids are not of ovarian origin.
- (d) Normal or moderately increased if associated with simple hirsutism or virilism, indicating possibly secondary hyperactivity of the adrenal cortex.

In patients with simple corpus luteum failure, as indicated by absence of pregnandiol in the urine and absence of secretory endometrium, the 17-ketosteroids are within normal limits.

Adrenocortical Deficiency (Addison's Disease). Women with Addison's disease excrete little or no 17-ketosteroid (0-1 mg. per twenty-four hours).² In men with Addison's disease the values are markedly diminished, but rarely are these substances absent (1-4 mg.), since some testicular function is usually present. The determination of urinary 17-ketosteroids is a valuable laboratory aid in the diagnosis of this condition, especially in women.

Hypothyroidism. In the majority of patients with untreated hypothyroidism, the urinary 17-ketosteroids are diminished and sometimes very low, usually ranging from 0.5 to 3.0 mg. in myxedema. The values are generally normal but occasionally moderately low in hyperthyroidism.

Nonendocrine Conditions. The 17-ketosteroids are often moderately low in chronic illnesses of all kinds, during the course of acute illnesses, including the common cold, in malnutrition,

anemias, anorexia nervosa, and after marked physical fatigue. Low values are commonly encountered in patients with hepatic disease.

INCREASED 17-KETOSTEROIDS

Very high values for total urinary 17-ketosteroids have been reported in adrenal cortical carcinoma, adrenal cortical hyperplasia and interstitial-cell tumors of the testis. Ovarian arrhenoblastoma is associated with normal or moderately increased values. Clinical manifestations and fractionation of the 17-ketosteroids are important in the differential diagnosis of these disorders.

Testicular Tumors. Very high 17-ketosteroid values have been reported in a patient with an interstitial-cell tumor of the testis, in whom the urinary androgen was chiefly androsterone sulfate.¹⁶ Chorionepithelioma and other embryonic tumors of the testis are associated with normal or decreased 17-ketosteroid values.

Adrenal Hyperfunction. Adrenal cortical hyperfunction may result in several clinical disorders, including Cushing's syndrome, the adrenogenital syndrome and a feminizing syndrome in males. These may be associated with simple hyperplasia or benign or malignant tumors of the adrenal cortex. The highest 17-ketosteroid values have been reported in patients with adrenal cortical carcinoma (up to 350 mg. or more per twenty-four hours), but there is considerable overlapping with cases of adrenal cortical hyperplasia.^{1,12} However, these two conditions may be distinguished by separating the urinary ketosteroids into alpha-alcoholic, beta-alcoholic and nonalcoholic fractions. The latter two (beta-alcoholic and nonalcoholic) are greatly increased in adrenal cortical carcinoma, but are normal or only slightly increased in hyperplasia. This finding, of high total 17-ketosteroids, with relatively high beta-alcoholic and non-alcoholic fractions, has been observed in carcinoma of the adrenal cortex associated with adrenogenital syndrome, Cushing's syndrome, and in a male patient with abnormal feminization.

In the few cases of arrhenoblastoma in which this determination has been made, the total 17-ketosteroid excretion has been normal or only slightly increased, despite the fact that this condition is accompanied by hirsutism and marked enlargement of the clitoris. It has been suggested that this tumor may secrete androgenic substances that are not excreted as 17-ketosteroids.¹¹

Following Endocrine Therapy. Administration of chorionic gonadotrophin may be followed by an increase in urinary 17-ketosteroids in the hypogonad male due to stimulation of the

testis, if the latter is capable of responding to stimulation. It has also been estimated that about 50 per cent of injected testosterone propionate is recoverable from the urine in 17-ketosteroid form. Administration of desoxycorticosterone acetate results in only a very slight increase in urinary 17-ketosteroids in patients with Addison's disease.²

ADRENAL CORTICAL HORMONES^{1, 4a, 5, 15a, 15b}

The adrenal cortex produces a number of steroid hormones which influence many physiologic functions, including electrolyte and water balance, capillary permeability, carbohydrate, protein and lipid metabolism, resistance to stress and toxins, growth, lactation, pigmentation, and gonadal and renal function. Twenty-eight steroid substances have been isolated from the adrenal cortex in pure crystalline form; six or seven of these have been found to be active, either in prolonging life in adrenalectomized animals or in preventing or relieving individual manifestations of deficiency. The most important of these may be grouped as follows:

Corticosterones. These include corticosterone, 11-dehydrocorticosterone and 11-dehydro 17-hydroxycorticosterone. All members of this group have in common an oxygen atom at carbon 11.

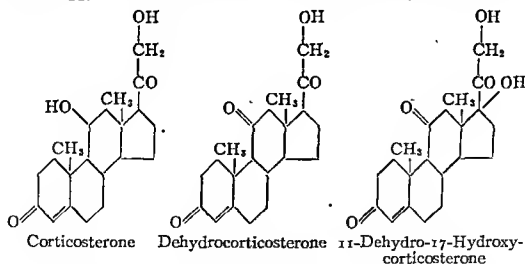


Fig. 28.

The corticosterones are concerned chiefly with carbohydrate metabolism (p. 22). They favor gluconeogenesis, probably chiefly from protein, and also appear to influence oxidation of glucose in the tissues. Corticosterone thus exerts a glycotropic or anti-insulin effect. The corticosterones are also highly effective in postponing muscular fatigue.

11-Desoxycorticosterone. This is structurally closely related

to progesterone, which has also been isolated from cortical extracts. The most striking effect of this hormone is upon salt and water metabolism. It causes retention of sodium, chloride and water in the body and increases the plasma volume. It lowers the concentration of potassium in the body fluids. When given in excessive amounts, it may produce edema, hypertension and congestive heart failure.

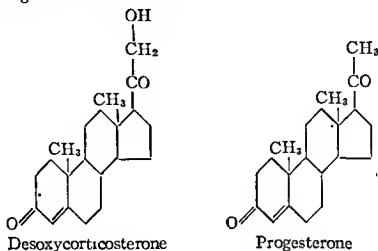


Fig. 29.

Desoxycorticosterone and the amorphous fraction are capable of restoring normal renal function in the adrenalectomized animal. Desoxycorticosterone is necessary for the normal growth of young adrenalectomized animals, but has no direct influence upon carbohydrate metabolism.

The Amorphous Fraction (Kendall). This fraction includes certain biologically active, unidentified compounds, present in the mother liquid of cortical extract after all of the crystalline steroids have been removed. It contains hormones that influence renal function but not salt and water metabolism. This fraction is very potent in its ability to maintain life in the adrenalectomized animal, but has little effect upon gluconeogenesis and muscular efficiency. It favorably influences growth in the young, adrenalectomized animal.

Sex Hormones. There are many observations that indicate that the adrenal cortex is intimately involved in the formation and metabolism of certain sex steroid hormones.

(a) Estrogens, androgens and progesterone have been isolated from the adrenal cortex.

(b) Estrogens and androgens are excreted by castrate animals.

(c) Excretion of estrogens, androgens or pregnandiol may be markedly increased in patients with adrenal cortical tumors or hyperfunction.

(d) The 17-ketosteroid excretion is markedly decreased in adrenal cortical hypofunction.

(e) The cortical and gonadal steroids are closely related chemically.

(f) The adrenal cortex undergoes hyperplasia during pregnancy.

(g) Animals withstand adrenalectomy during heat or pregnancy better than at other times.

(h) Progesterone lengthens the life span of adrenalectomized animals.

METHODS FOR EVALUATING ADRENAL CORTICAL FUNCTION

At the present time, no practical method is available for isolating the various cortical hormones from body fluids for clinical purposes. Consequently, methods for estimating adrenal cortical function are largely indirect in nature and involve either (a) tests of certain phases of carbohydrate, sodium chloride, potassium or water metabolism, or (b) quantitative determination of certain urinary steroids which originate at least in part in the adrenal cortex. The metabolic tests are often of great value clinically, and have been discussed in detail in the sections on carbohydrate (pp. 42, 46, 49) and sodium, chloride and potassium metabolism (pp. 242-245). They include the (1) glucose tolerance test, (2) insulin tolerance test, (3) salt excretion tests (Cutler) (Cantarow), (4) water excretion test (Kepler) and (5) basal metabolism determination. Additional information may be obtained regarding adrenal cortical function in some cases by the following studies.

Cortin-like Substances in Urine. A method for the recovery of "life-maintaining" steroids from the urine has been described by Weil and Browne,¹⁷ extracts of urine being injected into adrenalectomized mice. These substances were found to be increased in the urine of patients placed under conditions of stress, as after surgical operations, infections and exposure to cold.

Excretion of Estrogenic or Androgenic Substances. Some of the estrogenic and androgenic activity of normal urine is believed to be due to cortical steroids. In the discussion of estrogens and androgens, it was mentioned that some patients with adrenal cortical hyperfunction excrete increased amounts of estrogen and androgen. Conversely, excretion of these substances is decreased in the presence of adrenal cortical deficiency (Addison's disease).

Excretion of Pregnandiol. Increased amounts of pregnandiol or a pregnandiol-like substance have been found in the urine

of some patients with adrenal cortical hyperfunction. It is not known whether this is derived from progesterone produced by the adrenal cortex or from other adrenal steroids.

17-Ketosteroids. Some of the adrenal cortical hormones are excreted in the urine as 17-ketosteroids, particularly as beta-17-ketosteroids, *e.g.*, dehydroisoandrosterone. It is believed that in the female practically all and in the male about two thirds of the urinary 17-ketosteroids originate in the adrenal cortex. The determination of urinary 17-ketosteroids, including separation of the alpha and beta fractions, has therefore been widely employed in the clinical study of adrenal cortical function and often yields information of considerable diagnostic value. This subject has been discussed in detail elsewhere (pp. 558, 559).

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Chapter XXV

Outline of Chemical Abnormalities in Various Disorders

HYPERTHYROIDISM

1. Increased basal metabolism (p. 309).
2. Tendency toward fasting hyperglycemia (p. 26).
3. Decreased glucose tolerance (pp. 42, 38).
4. Glycosuria (p. 65).
5. Hypcholesterolemia (p. 159).
6. Decreased plasma phospholipid (p. 147).
7. Decreased plasma fat and fatty acid (p. 144).
8. Increased alimentary lipemia (p. 144).
9. Increased blood iodine (p. 218).
10. Increased iodine tolerance (p. 221).
11. Increased urine iodine excretion (p. 216).
12. Excessive creatinuria (pp. 123, 124).
13. Hypochlorhydria or achlorhydria (pp. 484, 486).
14. Increased urinary calcium (p. 185).
15. Decreased galactose tolerance (p. 52).

HYPOTHYROIDISM

1. Decreased basal metabolism (p. 311).
2. Tendency toward fasting hypoglycemia (p. 36).
3. Increased glucose tolerance (p. 47).
4. Hypercholesterolemia (p. 154).
5. Increased plasma fat and fatty acid (p. 144).
6. Decreased blood iodine (p. 218).
7. Creatine retention (p. 124).
8. Decreased urinary 17-ketosteroids (p. 558).
9. Carotinemia (p. 316).

HYPERPARATHYROIDISM

1. Hypercalcemia (p. 177).
2. Primary hypophosphatemia and secondary hyperphosphatemia (p. 192).
3. Increased urinary calcium (p. 185).
4. Increased urinary phosphate (p. 193).
5. Increased serum phosphatase activity (p. 199).

6. Decreased corpuscular ester phosphorus (p. 178).
7. Increased blood NPN, with renal functional impairment (p. 101).
8. Decreased blood and plasma volume (p. 241).
9. Dehydration and hemoconcentration (p. 241).
10. Hypochloremia (p. 241).
11. Increased parathyroid hormone in blood (p. 187).

HYPOPARATHYROIDISM

1. Hypocalcemia (p. 181).
2. Hyperphosphatemia (p. 191).
3. Decreased urinary calcium (p. 186).
4. Decreased urinary phosphate (p. 193).

HYPERPITUITARISM

1. Tendency toward increased basal metabolism (p. 309).
2. Tendency toward fasting hyperglycemia (p. 28).
3. Diminished glucose tolerance (p. 42).
4. Increased insulin resistance (p. 49).
5. Hyperlipemia (experimental) (p. 21).
6. Ketonuria (experimental) (p. 21).
7. Increased serum sodium (p. 234).
8. Increased serum chloride (p. 234).
9. Salt retention (p. 244).
10. Variable 17-ketosteroid excretion (p. 557).
11. Variable gonadotrophin excretion (p. 534).

HYPOPITUITARISM

1. Tendency toward decreased basal metabolism (p. 312).
2. Tendency toward fasting hypoglycemia (p. 36).
3. Increased glucose tolerance (p. 47).
4. Abnormal Staub-Traugott effect (p. 18).
5. Diminished epinephrine hyperglycemia (p. 18).
6. Increased insulin sensitivity (p. 49).
7. Decreased gonadotrophins (p. 537).
8. Decreased estrogen and androgen excretion (pp. 547, 554).
9. Decreased 17-ketosteroid excretion (p. 557).

HYPERCORTICOADRENALISM

1. Tendency toward increased basal metabolism (p. 310).
2. Tendency toward fasting hyperglycemia (p. 28).
3. Decreased sugar tolerance (p. 42).
4. Glycosuria (p. 66).
5. Salt and water retention (p. 244).
6. Increased urinary 17-ketosteroids (p. 559).
7. Variable pregnandiol excretion (p. 550).

HYPOCORTICOADRENALISM (ADDISON'S DISEASE)

1. Tendency toward decreased basal metabolism (p. 312).
2. Tendency toward fasting hypoglycemia (p. 35).
3. Increased sugar tolerance (p. 35).
4. Increased urinary sodium (p. 238).
5. Increased urinary chloride (p. 238).
6. Decreased urinary potassium (p. 238).
7. Decreased serum sodium (p. 238).
8. Decreased serum chloride (p. 238).
9. Increased serum potassium (p. 245).
10. Renal failure, with increased blood NPN (p. 239).
11. Decreased blood and plasma volume (p. 238).
12. Hemoconcentration and dehydration (p. 238).
13. Increased serum protein concentration (p. 238).
14. Increased blood O_2 capacity (p. 238).
15. Decreased 17-ketosteroid excretion (p. 558).
16. Increased insulin sensitivity (p. 49).

ACUTE HIGH INTESTINAL OBSTRUCTION

1. Hypochloremia (p. 235).
2. Decreased serum sodium and total base (p. 235).
3. Alkalosis (p. 284).
4. Ketonuria (pp. 163, 293).
5. Increased serum potassium (p. 246).
6. Hyperphosphatemia (p. 191).
7. Dehydration and hemoconcentration (p. 256).
8. Decreased blood and plasma volume (p. 94).
9. Hyperproteinemia (p. 256).
10. Hypocholesterolemia (p. 161).

PREGNANCY

1. Glycosuria (p. 517).
2. Decreased sugar tolerance (p. 517).
3. Excessive creatinuria (p. 83).
4. Decreased blood NPN and urea N (p. 516).
5. Increased blood undetermined N (p. 516).
6. Histidine in urine (p. 516).
7. Positive nitrogen balance (p. 516).
8. Variable blood urea clearance (p. 516).
9. Albuminuria (p. 515).
10. Decreased serum albumin (p. 515).
11. Increased plasma fatty acid (p. 517).
12. Increased plasma phospholipid (p. 517).
13. Hypercholesterolemia (p. 517).

14. Increased basal metabolism (p. 514).
15. Decreased serum calcium (p. 518).
16. Increased serum phosphatase activity (p. 519).
17. Decreased serum total base (p. 518).
18. Decreased plasma CO_2 combining power (p. 518).
19. Increased blood iodine (p. 218).
20. Increased urinary iodine (p. 217).
21. Increased blood and plasma volume (p. 514).
22. Hypochlorhydria (p. 486).
23. Decreased bilirubin excretion (p. 519).
24. Variable bromsulfalein excretion (p. 519).
25. Increased gonadotrophins (p. 531).
26. Increased estrogens (p. 545).
27. Increased pregnandiol (p. 549).

ANEMIA

PERNICIOUS ANEMIA

1. Decreased glucose tolerance (p. 43).
2. Diminished blood glycolysis (p. 54).
3. Increased plasma fat and fatty acid (p. 144).
4. Hypocholesterolemia (p. 157).
5. Decreased blood iron (p. 210).
6. Increased serum iron (p. 210).
7. Anoxemia (p. 301).
8. Achlorhydria, acbylia (p. 484).
9. Decreased blood volume (p. 252).
10. Variable plasma volume (p. 252).
11. Hyperbilirubinemia (pp. 438-440).
12. Diminished bilirubin excretion (p. 450).
13. Excessive urobilinuria (p. 446).

IDIOPATHIC HYPOCHROMIC ANEMIA

1. Decreased glucose tolerance (p. 43).
2. Increased plasma fat and fatty acid (p. 144).
3. Hypocholesterolemia (p. 157).
4. Decreased blood iron (p. 210).
5. Decreased serum iron (p. 210).
6. Anoxemia (p. 301).
7. Achlorhydria, hypochlorhydria (pp. 484, 486).
8. Variable blood and plasma volume (p. 252).

ACUTE HEMORRHAGIC ANEMIA

1. Anoxemia (p. 301).
2. Variable plasma volume (p. 252).

3. Decreased blood volume (p. 252).
4. Increased plasma fat and fatty acid (p. 144).
5. Hypercholesterolemia (p. 156).
6. Low blood iron (p. 210).

CHRONIC HEMORRHAGIC ANEMIA

1. Decreased glucose tolerance (p. 43).
2. Anoxemia (p. 301).
3. Increased plasma fat and fatty acid (p. 144).
4. Increased plasma phospholipid (p. 145).
5. Decreased blood iron (p. 210).
6. Increased plasma volume (p. 252).

HEMOLYTIC ANEMIA

1. Anoxemia (p. 301).
2. Increased plasma fat and fatty acid (p. 144).
3. Hypocholesterolemia (p. 157).
4. Low blood iron (p. 210).
5. Methemoglobinemia (p. 98).
6. Increased urobilinogen excretion (p. 446).
7. Hyperbilirubinemia (pp. 443, 438-440).

DIABETES MELLITUS

1. Glycosuria (p. 335).
2. Fasting hyperglycemia (p. 333).
3. Decreased glucose tolerance (p. 334).
4. Increased glucose in cerebrospinal fluid (p. 506).
5. Pentosuria (p. 68).
6. Decreased R.Q. (p. 332).
7. Increased plasma fat and fatty acid (pp. 144, 336).
8. Hypercholesterolemia (pp. 149, 336).
9. Hypocholesterolemia (pp. 150, 161).
10. Increased plasma phospholipid (p. 145).
11. Ketosis (pp. 163, 336).
12. Acidosis (pp. 279, 336, 338).
13. Decreased serum sodium (pp. 237, 339).
14. Hypochloremia (pp. 237, 338).
15. Increased blood NPN (p. 340).
16. Variable serum protein (p. 339).
17. Lipuria (p. 137).
18. Variable blood and plasma volume (p. 339).
19. Dehydration and hemoconcentration (p. 339).
20. Variable insulin sensitivity (p. 49).
21. Negative nitrogen balance (p. 85).

HYPOVITAMINOSIS D (RICKETS)

1. Hypophosphatemia (p. 191).
2. Variable serum calcium (p. 182).
3. Increased fecal excretion of calcium (p. 186).
4. Increased fecal excretion of phosphate (p. 193).
5. Decreased urinary calcium and phosphate (p. 186).
6. Increased serum phosphatase activity (p. 199).
7. Decreased corpuscular ester phosphorus (p. 198).

ICTERUS

EXTRAHEPATIC OBSTRUCTIVE JAUNDICE

1. Variable fasting blood sugar (p. 410).
2. Decreased glucose tolerance (p. 411).
3. Variable levulose tolerance (p. 415).
4. Variable galactose tolerance (p. 416).
5. Glycosuria (p. 66).
6. Decreased serum albumin (p. 423).
7. Decreased plasma prothrombin (pp. 425-427).
8. Increased blood fatty acid (p. 144).
9. Hypercholesterolemia (p. 428).
10. Hypocholesterolemia (p. 429).
11. Decreased plasma cholesterol esters (p. 429).
12. Increased fecal fat (pp. 135, 427).
13. Hypocalcemia (p. 184).
14. Increased serum phosphatase activity (pp. 460-463).
15. Increased blood iodine (p. 219).
16. Variable cephalin-cholesterol flocculation (p. 424).
17. Hyperbilirubinemia (p. 440).
18. Bilirubinuria (p. 443).
19. Variable fecal urobilinogen (p. 445).
20. Variable urobilinuria (p. 448).
21. Variable blood bile acids (p. 453).
22. Positive direct van den Bergh reaction (p. 440).
23. Impaired bilirubin excretion (p. 449).
24. Impaired bromsulfalein excretion (p. 458).
25. Bile acids in urine (p. 453).
26. Changes in bile (pp. 467-472).

HEPATOCELLULAR JAUNDICE

1. Variable fasting blood sugar (p. 410).
2. Decreased glucose tolerance (p. 411).
3. Variable levulose tolerance (p. 415).
4. Decreased galactose tolerance (p. 416).
5. Glycosuria (p. 66).

6. Decreased serum albumin (p. 422).
7. Increased serum globulin (pp. 422-425).
8. Variable blood lactic acid (p. 417).
9. Diminished lactate tolerance (p. 418).
10. Variable plasma fibrinogen (p. 421).
11. Decreased plasma prothrombin (pp. 425-427).
12. Positive globulin reactions (pp. 423-425).
13. Tendency toward decreased blood urea N (p. 420).
14. Tendency toward increased blood uric acid (p. 421).
15. Increased blood guanidine (p. 421).
16. Tyrosinuria (p. 419).
17. Impaired hippuric acid synthesis (p. 456).
18. Decreased epinephrine hyperglycemia (p. 413).
19. Increased plasma fatty acid (p. 144).
20. Hypercholesterolemia (p. 429).
21. Hypocholesterolemia (pp. 158, 429).
22. Decreased plasma cholesterol esters (pp. 158, 429).
23. Hypochloremia (p. 241).
24. Increased serum phosphatase activity (pp. 460-463).
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NORMAL CHEMICAL STANDARDS

(All values expressed in mg. per 100 cc. unless otherwise stated)

WHOLE BLOOD

Hemoglobin (Gm. per 100 cc.) (average).....	15.6
Glucose { Polin-Wu.....	80-120
{ Benedict.....	60-110
Nonprotein nitrogen.....	25-35 (16-40)
Urea nitrogen.....	9-17 (5-23)
Creatinine.....	1-2
Uric acid.....	2-4
Amino acid nitrogen.....	5-8
Ammonia nitrogen.....	0.1-0.2
Undetermined nitrogen.....	4-18
Iron.....	52
.....	24-55
.....	6-20
.....	72-100 cc.
O ₂ capacity (volumes per 100 cc.).....	450-500
O ₂ content (arterial) (volumes per 100 cc.).....	16-24
.....	15-23

30 minutes.	13-24%
60 minutes	9-17%
120 minutes	3-10%
Urobilinogen excretion (mg. in 24 hours) {	
urine.	1-4
feces.	40-280
Hippuric acid test (oral) (4 hour excretion).	3 Gm.
Intravenous bilirubin tolerance (in blood at end	
of 4 hours)	0-6%
Basal metabolic rate	+15-15
Maximum blood urea clearance (average)	75 cc.
Standard blood urea clearance (average).	54 cc.
Plasma diodrast clearance (average)	700 cc.
Plasma hippuran clearance (average)	700 cc.
Plasma inulin clearance (average).	125 cc.
Respiratory quotient (fasting)	0.82

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